

U.S. PATENT APPLICATION

Inventor(s): *Thomas Antonsson*
 David Gustafsson
 Kurt-Jürgen Hoffmann
 Jan-Erik Nyström
 Henrik Sørensen
 Mikael Sellén

Invention: ***PRODRUGS OF THROMBIN INHIBITORS***

NIXON & VANDERHYE P.C.
ATTORNEYS AT LAW
1100 NORTH GLEBE ROAD
8TH FLOOR
ARLINGTON, VIRGINIA 22201-4714
(703) 816-4000
Telecopier (703) 816-4100

SPECIFICATION

Prodrugs of Thrombin Inhibitors

Field of the Invention

5 This invention relates to pharmaceutically useful prodrugs of pharmaceutically active compounds, which active compounds are, in particular, competitive inhibitors of trypsin-like serine proteases, especially thrombin, the use of the prodrugs as medicaments, pharmaceutical compositions containing them and synthetic routes to their
10 production.

Background

Blood coagulation is the key process involved in both haemostasis (ie the
15 prevention of blood loss from a damaged vessel) and thrombosis (ie the formation of a blood clot in a blood vessel, sometimes leading to vessel obstruction).

Coagulation is the result of a complex series of enzymatic reactions. One
20 of the ultimate steps in this series of reactions is the conversion of the proenzyme prothrombin to the active enzyme thrombin.

Thrombin is known to play a central role in coagulation. It activates platelets, leading to platelet aggregation, converts fibrinogen into fibrin
25 monomers, which polymerise spontaneously into fibrin polymers, and activates factor XIII, which in turn crosslinks the polymers to form insoluble fibrin. Furthermore, thrombin activates factor V and factor VIII leading to a "positive feedback" generation of thrombin from prothrombin.

30 By inhibiting the aggregation of platelets and the formation and

crosslinking of fibrin, effective inhibitors of thrombin would therefore be expected to exhibit antithrombotic activity. In addition, antithrombotic activity would be expected to be enhanced by effective inhibition of the positive feedback mechanism.

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Prior Art

The development of low molecular weight inhibitors of thrombin has been described by Claesson in Blood Coagul. Fibrin. (1994) 5, 411.

10

Blombäck *et al* (in J. Clin. Lab. Invest. 24, suppl. 107, 59, (1969)) reported thrombin inhibitors based on the amino acid sequence situated around the cleavage site for the fibrinogen A α chain. Of the amino acid sequences discussed, these authors suggested the tripeptide sequence Phe-Val-Arg would be the most effective inhibitor.

15

Low molecular weight peptide-based thrombin inhibitors have subsequently been disclosed in, for example, US Patent N° 4,346,078; International Patent Applications WO 93/11152, WO 94/29336, WO 93/18060 and WO 95/01168; and European Patent Applications 648 780, 468 231, 559 046, 641 779, 185 390, 526 877, 542 525, 195 212, 362 002, 364 344, 530 167, 293 881, 686 642 and 601 459.

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More recently, thrombin inhibitors based on peptide derivatives have been disclosed in European Patent Application 0 669 317 and International Patent Applications WO 95/23609, WO 95/35309, WO 96/25426 and WO 94/29336.

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In particular, the latter application discloses the peptide derivatives R^aOOC-CH₂-(R)Cgl-Aze-Pab-H, wherein R^a represents H, benzyl or C₁₋₆

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alkyl.

Although these active compounds are known to exhibit significant antithrombin activity, it would be beneficial to improve their pharmacokinetic properties both after oral and parenteral administration. Examples of pharmacokinetic properties which it is desirable to improve include:

- 10 (a) providing an improved absorption from the gastro-intestinal tract, with a view to reducing intra- and/or inter-individual variability in relation to the bioavailability of the active compounds;
- 15 (b) flattening the plasma concentration time profile (ie reducing the peak/trough ratio in the plasma concentration over the dosing interval), with a view to reducing the risk of falling outside the therapeutic interval and the side effects caused by a concentration peak which is too high (eg bleeding), and those caused by one which is too low (eg thrombus formation); and
- 20 (c) increasing the duration of action of the active compounds.

Moreover, oral and parenteral administration of active thrombin inhibitors may lead to undesirable local bleeding (eg in the intestinal lumen or subcutaneously) as a result of a high local concentration,

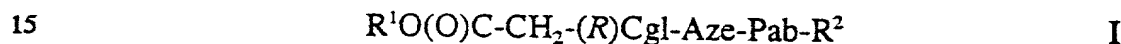
25 Finally, orally administered active thrombin inhibitors which also inhibit trypsin and other serine proteases in the gastrointestinal tract may exhibit additional side effects, including indigestion (eg if trypsin is inhibited in the intestinal lumen).

Although certain N-benzylloxycarbonyl derivatives of the aforementioned active compounds are also disclosed as thrombin inhibitors in International Patent Application WO 94/29336, that these derivatives may be useful as prodrugs is not mentioned. In fact, WO 94/29336 makes no mention of
 5 suitable prodrugs of the active compounds.

We have found that the above problems may be solved by administering compounds according to the present invention which, whilst inactive *per se*, upon oral and/or parenteral administration are metabolised in the body
 10 to form active thrombin inhibitors, including those mentioned above.

Disclosure of the Invention

According to the invention there is provided a compound of formula I,



wherein

R^1 represents $-R^3$ or $-A^1C(O)N(R^4)R^5$ or $-A^1C(O)OR^4$;

A^1 represents C_{1-5} alkylene;

R^2 (which replaces one of the hydrogen atoms in the amidino unit of
 20 Pab-H) represents OH, $OC(O)R^6$, $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$;
 R^3 represents H, C_{1-10} alkyl, or C_{1-3} alkylphenyl (which latter group is optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or halogen);

R^4 and R^5 independently represent H, C_{1-6} alkyl, phenyl, 2-naphthyl or, when R^1 represents $-A^1C(O)N(R^4)R^5$, together with the nitrogen atom to
 25 which they are attached represent pyrrolidinyl or piperidinyl;

R^6 represents C_{1-17} alkyl, phenyl or 2-naphthyl (all of which are optionally substituted by C_{1-6} alkyl or halogen);

R^7 represents 2-naphthyl, phenyl, C_{1-3} alkylphenyl (which latter three groups are optionally substituted by C_{1-6} alkyl, C_{1-6} alkoxy, nitro or
 30 halogen), or C_{1-12} alkyl (which latter group is optionally substituted by C_{1-6}

alkoxy, C₁₋₆ acyloxy or halogen);

R⁸ represents H or C₁₋₄ alkyl; and

R⁹ represents 2-naphthyl, phenyl, C₁₋₆ alkoxy or C₁₋₈ alkyl (which latter group is optionally substituted by halogen, C₁₋₆ alkoxy or C₁₋₆ acyloxy);

5 provided that when R¹ represents R³, R³ represents benzyl, methyl, ethyl, *n*-butyl or *n*-hexyl and R² represents C(O)OR⁷, then R⁷ does not represent benzyl;

or a pharmaceutically-acceptable salt thereof (hereinafter referred to as "the compounds of the invention").

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The compounds of the invention may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

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The compounds of the invention may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism.

All diastereoisomers may be separated using conventional techniques, eg chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the compounds using conventional, eg fractional crystallisation or HPLC,

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techniques. Alternatively the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereomeric derivatives by conventional means (eg HPLC, 25 chromatography over silica). All stereoisomers are included within the scope of the invention.

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According to a further aspect of the invention there is provided the use of a compound of formula I, as hereinbefore defined but without the proviso, as a prodrug.

- Alkyl groups which R^3 , R^4 , R^5 , R^6 , R^7 and R^9 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched, be cyclic or partially cyclic, be saturated or unsaturated, be interrupted by oxygen and/or be substituted or terminated by OH, provided that the OH group is not attached to an sp^2 carbon atom or a carbon atom which is adjacent to an oxygen atom.

By "partially cyclic alkyl groups" we mean groups such as CH_2Ch .

- Alkyl groups which R^8 may represent, and R^3 , R^6 and R^7 may be substituted by, may be linear or, when there are a sufficient number of carbon atoms, be branched, be saturated or unsaturated and/or be interrupted by oxygen.
- The alkyl portion of alkylphenyl groups which R^3 and R^7 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

- Alkylene groups which A^1 may represent may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

- Alkoxy groups which R^9 may represent, and R^3 , R^7 and R^9 may be substituted by, may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Acyloxy groups which R^7 and R^9 may be substituted by may be linear or, when there are a sufficient number of carbon atoms, be branched and/or be saturated or unsaturated.

Abbreviations are listed at the end of this specification.

According to a further aspect of the invention there is provided a compound of formula I, as hereinbefore defined, with the additional provisos that:

- (a) R^1 does not represent $-A^1C(O)OR^4$;
- (b) R^4 and R^5 do not independently represent H;
- (c) R^6 does not represent C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

According to a further aspect of the invention there is provided a compound of formula I, wherein:

- (a) R^1 represents $-A^1C(O)OR^4$;
- (b) R^4 and R^5 independently represent H;
- (c) R^6 represents C_{1-17} alkyl, when R^2 represents $OC(O)R^6$.

When R^1 represents $-A^1C(O)N(R^4)R^5$, preferred compounds of the invention include those wherein:

A^1 represents C_{1-3} alkylene;

R^4 represents H or C_{1-6} alkyl;

R^5 represents C_{1-6} alkyl or C_{4-6} cycloalkyl; or those wherein R^4 and R^5 together represent pyrrolidinyl.

When R^1 represents $-A^1C(O)OR^4$, preferred compounds of the invention include those wherein:

A^1 represents C_{1-5} alkylene;

R^4 represents C_{1-6} alkyl.

When R^1 represents R^3 , preferred compounds of the invention include those wherein R^3 represents H, C_{1-10} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be

branched and/or be partially cyclic or cyclic), or C_{1-3} alkylphenyl (which latter groups is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, be branched).

- 5 Preferred compounds of the invention include those wherein R^2 represents OH, $OC(O)R^6$ (wherein, in the latter case, R^6 represents optionally substituted phenyl or C_{1-17} alkyl (which latter group may be linear or, when there are a sufficient number of carbon atoms, may be branched, be cyclic or partially cyclic, and/or be saturated or unsaturated)), $C(O)OR^7$
 10 (wherein, in the latter case, R^7 represents optionally substituted phenyl, C_{1-12} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched, cyclic or partially cyclic, and/or saturated or unsaturated), or C_{1-3} alkylphenyl (which latter group is optionally substituted, may be linear or,
 15 when there are a sufficient number of carbon atoms, may be branched)), or $C(O)OCH(R^8)OC(O)R^9$ (wherein, in the latter case, R^8 represents H or methyl, and R^9 represents phenyl, or C_{1-8} alkyl (which latter group is optionally substituted, may be linear or, when there are a sufficient number of carbon atoms, may be branched and/or cyclic or partially
 20 cyclic)).

More preferred compounds of the invention include those wherein:

- R^1 represents H, linear C_{1-10} alkyl, branched C_{3-10} alkyl, partially cyclic C_{4-10} alkyl, C_{4-10} cycloalkyl, optionally substituted linear C_{1-3} alkylphenyl,
 25 optionally substituted branched C_3 alkylphenyl, $-A^1C(O)N(R^4)R^5$ (wherein, in the latter case, A^1 represents C_{1-3} alkylene, and R^4 represents H or C_{1-3} alkyl and R^5 represents C_{2-6} alkyl or C_{3-6} cycloalkyl, or R^4 and R^5 together represent pyrrolidinyl), or $-A^1C(O)OR^4$ (wherein, in the latter case, A^1 represents C_{1-5} alkylene and R^4 represents C_{1-4} alkyl);
 30 R^2 represents OH, $OC(O)R^6$ (wherein, in the latter case, R^6 represents

optionally substituted phenyl, linear C₁₋₄ alkyl, branched C₃₋₄ alkyl or *cis*-oleyl), C(O)OR⁷ (wherein, in the latter case, R⁷ represents optionally substituted and/or optionally unsaturated linear C₁₋₄ alkyl or optionally substituted and/or optionally unsaturated branched C₃₋₄ alkyl, optionally substituted phenyl, or optionally substituted linear C₁₋₃ alkylphenyl or optionally substituted branched C₃ alkylphenyl) or C(O)OCH(R⁸)OC(O)R⁹ (wherein, in the latter case, R⁸ represents H or methyl and R⁹ represents phenyl, C₅₋₇ cycloalkyl, linear C₁₋₆ alkyl, branched C₃₋₆ alkyl or partially cyclic C₇₋₈ alkyl).

10

Particularly preferred compounds of the invention include those wherein: R¹ represents linear C₁₋₆ alkyl, C₆₋₁₀ cycloalkyl, or optionally substituted linear C₁₋₃ alkylphenyl;

R² represents OH, OC(O)R⁶ (wherein, in the latter case, R⁶ represents linear C₁₋₃ alkyl or branched C₃ alkyl), C(O)OR⁷ (wherein, in the latter case, R⁷ represents optionally substituted linear C₁₋₄ alkyl or optionally substituted branched C₃₋₄ alkyl, optionally substituted linear C₁₋₃ alkylphenyl or branched C₃ alkylphenyl) or C(O)OCH(R⁸)OC(O)R⁹ (wherein, in the latter case, R⁸ represents H and R⁹ represents C₅₋₇ cycloalkyl, linear C₁₋₆ alkyl or partially cyclic C₇₋₈ alkyl).

When R¹ represents R³ and R³ represents optionally substituted C₁₋₃ alkylphenyl, preferred optional substituent include C₁₋₆ alkyl (especially methyl).

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When R² represents C(O)OR⁷ and R⁷ represents optionally substituted C₁₋₁₂ alkyl, preferred optional substituents include halogen (especially chloro) and C₁₋₆ alkoxy (especially methoxy).

When R² represents C(O)OR⁷ and R⁷ represents optionally substituted

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phenyl, preferred optional substituents include C₁₋₆ alkyl (especially methyl), C₁₋₆ alkoxy (especially methoxy) and halogen (especially chloro).

When R² represents C(O)OR⁷ and R⁷ represents optionally substituted C₁₋₃ alkylphenyl, preferred optional substituents include nitro.

Preferred compounds of the invention include the compounds of Examples 1 to 68.

10 More preferred compounds of the invention include:

EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂;

*n*PrOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂;

*t*BuOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂;

EtOOCCH₂-(R)Cgl-Aze-Pab-COOEt;

15 EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Bu;

PrIC(O)CH₂CH₂CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z;

ChNHC(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z;

(*n*Pr)₂NC(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCC(CH₃)₃;

EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCC(CH₃)₃;

20 EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCCH₃;

MeOOCCH₂-(R)Cgl-Aze-Pab-OOCPh;

MeOOCCH₂-(R)Cgl-Aze-Pab-OH;

EtOOCCH₂-(R)Cgl-Aze-Pab-OH;

BnOOCCH₂-(R)Cgl-Aze-Pab-OH;

25 *n*PrOOCCH₂-(R)Cgl-Aze-Pab-Z;

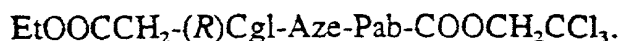
*n*PrOOCCH₂-(R)Cgl-Aze-Pab-OH;

*i*PrOOCCH₂-(R)Cgl-Aze-Pab-OH;

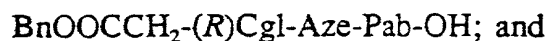
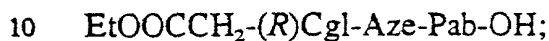
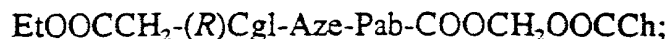
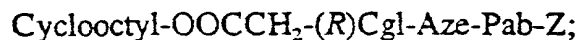
*t*BuOOCCH₂-(R)Cgl-Aze-Pab-OH;

(*n*Pr)₂NCOCH₂OOCCH₂-(R)Cgl-Aze-Pab-OH;

- $\text{ChNHCOCH}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-OH};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-OAc};$
 $\text{HOOCCH}_2-(R)\text{Cgl-Aze-Pab-OH};$
 $\text{HOOCCH}_2-(R)\text{Cgl-Aze-Pab-O-}i\text{-cis-Oleyl};$
5 $\text{Cyclooctyl-OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $i\text{BuCH}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $(2\text{-Me})\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $\text{ChCH}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $\text{ChOOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
10 $\text{PhC(Me)}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $(\text{Me})_2\text{CHC(Me)}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-OMe)};$
 $\text{ChCH}_2\text{OOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-OMe)};$
 $(2\text{-Me})\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-OMe)};$
15 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-Me)};$
 $\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-Me)};$
 $\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COO-}n\text{Bu};$
 $i\text{PrOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{CH=CH}_2;$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COO-}i\text{Bu};$
20 $\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COO-}n\text{Pr};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{OOCCh};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{OOCCH}_2\text{Ch};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH(Me)OOCPh};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{OOCPh};$
25 $\text{BnOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH(Me)OAc};$
 $\text{EtOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{OAc};$
 $i\text{BuOOCCH}_2-(R)\text{Cgl-Aze-Pab-COOCH}_2\text{OAc};$
 $\text{MeOOC-C(=CHEt)CH}_2\text{-OOCCH}_2-(R)\text{Cgl-Aze-Pab-Z};$
 $\text{Men-OOCCH}_2-(R)\text{Cgl-Aze-Pab-COOPh(4-OMe)}; \text{ and}$



Particularly preferred compounds of the invention include:



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Preparation

According to the invention there is also provided a process for the preparation of compounds of formula I which comprises:

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(a) Preparation of a compound of formula I wherein R^2 represents OH by reaction of a corresponding compound of formula I, wherein R^2 represents $\text{OC(O)}R^6$ and R^6 is as hereinbefore defined, with an alkoxide base (eg an alkali metal alkoxide), for example at room temperature in the presence
25 of an appropriate organic solvent (eg THF).

30

(b) Preparation of a compound of formula I wherein R^2 represents OH by reaction of a corresponding compound of formula I wherein R^2 represents $\text{C(O)}\text{OR}^7$ and R^7 is as hereinbefore defined with hydroxylamine, or an acid addition salt thereof, for example at room temperature in the presence of

a suitable base (eg potassium carbonate or triethylamine) and an appropriate organic solvent (eg THF or EtOH).

- (c) Preparation of a compound of formula I by reaction of a corresponding
5 compound of formula II,



wherein R^2 is as hereinbefore defined with a compound of formula III,



- wherein L^1 represents a leaving group, for example halide (eg bromide)
10 or alkylsulphonate (eg trifluoromethylsulphonate) and R^1 is as hereinbefore defined, for example between room and elevated temperature (eg 40°C) in the presence of a suitable base (eg potassium carbonate) and an appropriate organic solvent (eg THF, DMF or acetonitrile).

- 15 (d) Preparation of a compound of formula I wherein R^1 represents H and R^2 represents OH or C(O)OR^7 and R^7 is as hereinbefore defined by reaction of a corresponding compound of formula I wherein R^1 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, and R^2 represents OH or C(O)OR^7 , with a suitable base (eg an alkali metal alkoxide or hydroxide), for example at
20 room temperature in the presence of an appropriate organic solvent (eg water or MeOH).

- (e) Preparation of a compound of formula I wherein R^2 represents OC(O)R^6 and R^6 is as hereinbefore defined, by reaction of a corresponding
25 compound of formula I wherein R^2 represents OH, with a compound of formula IV,



or a compound of formula V,



- 30 wherein Hal represents Cl or Br and, in both cases, R^6 is as hereinbefore

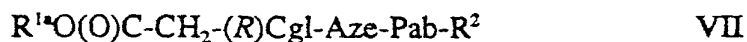
defined, for example at room temperature in the presence of a suitable base (eg triethylamine, pyridine or DMAP) and an appropriate organic solvent (eg methylene chloride or THF).

- 5 (f) Preparation of a compound of formula I wherein R^1 represents H and R^2 represents $OC(O)R^6$, and R^6 is as hereinbefore defined, by reaction of a corresponding compound of formula VI



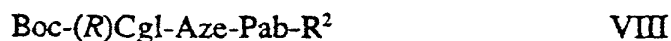
- wherein P^1 represents an acid labile ester protecting group (eg *t*Bu or Bn),
 10 and R^2 represents $OC(O)R^6$, wherein R^6 is as hereinbefore defined, with a suitable acid (eg TFA), for example at room temperature in the presence of an appropriate organic solvent (eg methylene chloride).

- (g) Preparation of a compound of formula I wherein R^1 represents R^3 , R^3
 15 represents C_{1-10} alkyl or C_{1-3} alkylphenyl, R^2 represents OH or $C(O)OR^7$, and R^7 is as hereinbefore defined, by a trans-esterification of a corresponding compound of formula VII,



- wherein R^{1a} represents a C_{1-10} alkyl or C_{1-3} alkylphenyl group other than
 20 that being formed and R^2 is as hereinbefore defined or an alternative labile alkyl substituent, under conditions which are well known to those skilled in the art.

- Compounds of formula II may be prepared by deprotection of a compound
 25 of formula VIII,



wherein R^2 is as hereinbefore defined, under conditions which are well known to those skilled in the art.

- 30 Compounds of formula VI and VII may be prepared analogously to those

methods described hereinbefore for preparation of compounds of formula I, in which R^1 represents R^3 and R^3 represents C_{1-10} alkyl or C_{1-3} alkylphenyl.

- 5 Compounds of formula VIII may be prepared by reaction of a compound of formula IX,



- wherein R^2 is as hereinbefore defined with Boc-Cgl-Aze-OH, for example at room temperature in the presence of a suitable coupling system (eg EDC), an appropriate base (eg DMAP) and a suitable organic solvent (eg dichloromethane or acetonitrile).

- Compounds of formula VIII, wherein R^2 represents OH may be prepared by reaction of a corresponding compound of formula VIII, wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$, with hydroxylamine, or an acid addition salt thereof, for example at room temperature in the presence of a suitable base (eg potassium carbonate or triethylamine) and an appropriate organic solvent (eg THF or EtOH).

- 20 Compounds of formula VIII, wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$, may be prepared by reaction of Boc-(R)Cgl-Aze-Pab-H with a compound of formula X,

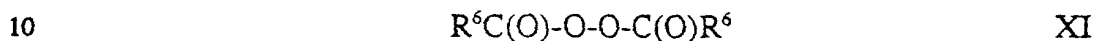


- wherein L^2 represents a leaving group (eg halogen or phenolate) and R^{2a} represents R^7 or $-CH(R^8)OC(O)R^9$ and R^7 , R^8 and R^9 are as hereinbefore defined, for example at or below room temperature in the presence of a suitable base (eg NaOH) and an appropriate organic solvent (eg THF).

- Compounds of formula VIII, wherein R^2 represents $OC(O)R^6$ may alternatively be prepared by reaction of a corresponding compound of

formula VIII, wherein R^2 represents OH with a compound of formula IV as hereinbefore defined or a compound of formula V as hereinbefore defined, for example at room temperature in the presence of a suitable base (eg triethylamine, pyridine or DMAP) and an appropriate organic solvent (eg methylene chloride or THF).

Compounds of formula VIII wherein R^2 represents $OC(O)R^6$ may alternatively be prepared by reaction of Boc-(R)Cgl-Aze-Pab-H with a compound of formula XI,



wherein R^6 is as hereinbefore defined, for example at room temperature in the presence of an appropriate organic solvent (eg THF).

Compounds of formula VIII wherein R^2 represents OH may be prepared by reaction of a corresponding compound of formula VIII, wherein R^2 represents $OC(O)R^6$ and R^6 is as hereinbefore defined with a suitable base (eg an alkali metal alkoxide), for example at room temperature in the presence of an appropriate solvent (eg THF).

Compounds of formula IX are well known in the literature or may be prepared using methods analogous to those described hereinbefore. For example, compounds of formula IX wherein R^2 represents $C(O)OR^7$ or $C(O)OCH(R^8)OC(O)R^9$ and R^7 , R^8 and R^9 are as hereinbefore defined may be prepared by reaction of H-Pab-H, or a protected derivative thereof, with a compound of formula X, as hereinbefore defined, for example at or below room temperature in the presence of a suitable base (eg NaOH) and an appropriate organic solvent (eg THF).

Boc-(R)Cgl-Aze-Pab-H may be prepared by reaction of H-Pab-H, or a protected derivative thereof, with Boc-Cgl-Aze-OH, for example as

hereinafter.

The use of protecting groups is fully described in 'Protective Groups in Organic Chemistry', edited by J W F McOmie, Plenum Press (1973), and
5 'Protective Groups in Organic Synthesis', 2nd edition, T W Greene & P G M Wutz, Wiley-Interscience (1991).

Medical and pharmaceutical use

10 The compounds of the invention are useful because they are metabolised in the body to form compounds which possess pharmacological activity. They are therefore indicated as pharmaceuticals and in particular as prodrugs.

15 In particular, the compounds of the invention, although they are inactive to thrombin *per se*, are metabolised in the body to form potent inhibitors of thrombin, for example as demonstrated in the test described below.

By "the compounds of the invention are inactive to thrombin *per se*" we
20 mean that they exhibit an $IC_{50}TT$ value, as determined in Test A below, of greater than $1 \mu M$.

The compounds of the invention are thus expected to be useful in those conditions where inhibition of thrombin is required.

25

The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of thrombosis and hypercoagulability in blood and tissues of animals including man.

30 It is known that hypercoagulability may lead to thrombo-embolic diseases.

Thrombo-embolic diseases which may be mentioned include activated protein C resistance, such as the factor V-mutation (factor V Leiden), and inherited or acquired deficiencies in antithrombin III, protein C, protein S, heparin cofactor II. Other conditions known to be associated with hypercoagulability and thrombo-embolic disease include circulating antiphospholipid antibodies (Lupus anticoagulant), homocysteinemia, heparin induced thrombocytopenia and defects in fibrinolysis. The compounds of the invention are thus indicated both in the therapeutic and/or prophylactic treatment of these conditions.

10

The compounds of the invention are further indicated in the treatment of conditions where there is an undesirable excess of thrombin without signs of hypercoagulability, for example in neurodegenerative diseases such as Alzheimer's disease.

15

Particular disease states which may be mentioned include the therapeutic and/or prophylactic treatment of venous thrombosis and pulmonary embolism, arterial thrombosis (eg in myocardial infarction, unstable angina, thrombosis-based stroke and peripheral arterial thrombosis) and systemic embolism usually from the atrium during arterial fibrillation or from the left ventricle after transmural myocardial infarction.

20

Moreover, the compounds of the invention are expected to have utility in prophylaxis of re-occlusion (ie thrombosis) after thrombolysis, percutaneous trans-luminal angioplasty (PTA) and coronary bypass operations; the prevention of re-thrombosis after microsurgery and vascular surgery in general.

25

Further indications include the therapeutic and/or prophylactic treatment of disseminated intravascular coagulation caused by bacteria, multiple

30

trauma, intoxication or any other mechanism; anticoagulant treatment when blood is in contact with foreign surfaces in the body such as vascular grafts, vascular stents, vascular catheters, mechanical and biological prosthetic valves or any other medical device; and anticoagulant
5 treatment when blood is in contact with medical devices outside the body such as during cardiovascular surgery using a heart-lung machine or in haemodialysis.

In addition to its effects on the coagulation process, thrombin is known to
10 activate a large number of cells (such as neutrophils, fibroblasts, endothelial cells and smooth muscle cells). Therefore, the compounds of the invention may also be useful for the therapeutic and/or prophylactic treatment of idiopathic and adult respiratory distress syndrome, pulmonary
15 fibrosis following treatment with radiation or chemotherapy, septic shock, septicemia, inflammatory responses, which include, but are not limited to, edema, acute or chronic atherosclerosis such as coronary arterial disease, cerebral arterial disease, peripheral arterial disease, reperfusion damage, and restenosis after percutaneous trans-luminal angioplasty (PTA).

20 Compounds of the invention that inhibit trypsin and/or thrombin may also be useful in the treatment of pancreatitis.

According to a further aspect of the present invention, there is provided a method of treatment of a condition where inhibition of thrombin is
25 required which method comprises administration of a therapeutically effective amount of a compound of formula I as defined above, or a pharmaceutically acceptable salt thereof, to a person suffering from, or susceptible to such a condition.

30 The compounds of the invention will normally be administered orally,

buccally, rectally, dermally, nasally, tracheally, bronchially, by any other parenteral route or *via* inhalation, in the form of pharmaceutical preparations comprising the prodrug either as a free base, or a pharmaceutical acceptable non-toxic organic or inorganic acid addition salt, in a pharmaceutically acceptable dosage form. Depending upon the disorder and patient to be treated and the route of administration, the compositions may be administered at varying doses.

The compounds of the invention may also be combined and/or co-administered with any antithrombotic agent with a different mechanism of action, such as the antiplatelet agents acetylsalicylic acid, ticlopidine, clopidogrel, thromboxane receptor and/or synthetase inhibitors, fibrinogen receptor antagonists, prostacyclin mimetics and phosphodiesterase inhibitors and ADP-receptor (P_2T) antagonists.

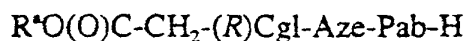
The compounds of the invention may further be combined and/or co-administered with thrombolytics such as tissue plasminogen activator (natural or recombinant), streptokinase, urokinase, prourokinase, anisolated streptokinase plasminogen activator complex (ASPAC), animal salivary gland plasminogen activators, and the like, in the treatment of thrombotic diseases, in particular myocardial infarction.

According to a further aspect of the invention there is thus provided a pharmaceutical formulation including a compound of formula I as hereinbefore defined, or a pharmaceutically acceptable salt thereof, in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

Suitable daily doses of the compounds of the invention in therapeutical treatment of humans are about 0.001-100mg/kg body weight at peroral administration and 0.001-50mg/kg body weight at parenteral

administration.

The compounds of the invention have the advantage that they may have improved pharmacokinetic properties, such as those identified
5 hereinbefore, both after oral and parenteral administration, when compared with compounds of formula:



wherein R^a is as hereinbefore defined, and in particular the compound wherein R^a represents H.

10

The compounds of the invention are inactive to thrombin, trypsin and other serine proteases. The compounds thus remain inactive in the gastrointestinal tract and the potential complications experienced by orally administered anticoagulants which are active *per se*, such as bleeding and
15 indigestion resulting from inhibition of trypsin, may thus be avoided.

Furthermore, local bleeding associated with and after parenteral administration of an active thrombin inhibitor may be avoided by using the compounds of the invention.

20

The compounds of the invention may also have the advantage that they may be more efficacious than, be less toxic than, be longer acting than, have a broader range of activity than, produce fewer side effects than, be more easily absorbed than, or that they may have other useful
25 pharmacological properties over, compounds known in the prior art.

Biological Tests

Test A

Determination of Thrombin clotting Time (TT)

- 5 Human thrombin (T 6769, Sigma Chem Co, final concentration of 1.4 NIH units/mL) in buffer solution, pH 7.4, 100 μ L, and inhibitor solution, 100 μ L, were incubated for one min. Pooled normal citrated human plasma, 100 μ L, was then added and the clotting time measured in an automatic device (KC 10, Amelung).

10

The clotting time in seconds was plotted against the inhibitor concentration, and the $IC_{50}TT$ was determined by interpolation.

- 15 $IC_{50}TT$ is the concentration of inhibitor that doubles the thrombin clotting time for human plasma.

Test B

Determination of thrombin time in plasma *ex vivo*

- 20 The inhibition of thrombin after oral or parenteral administration of the compounds of the invention were examined in conscious rats which, one or two days prior to the experiment, were equipped with a catheter for blood sampling from the carotid artery. On the experimental day, the compound, dissolved in ethanol:Solutol™:water (5:5:90), was administered and blood samples were withdrawn at fixed times into plastic tubes
- 25 containing 1 part sodium citrate solution (0.13 mol per L.) and 9 parts of blood. The tubes were centrifuged to obtain platelet poor plasma. The plasma was used for determination of thrombin time as described below.

- 30 The citrated rat plasma, 100 μ L, was diluted with a saline solution, 0.9%, 100 μ L, and plasma coagulation was started by the addition of human

thrombin (T 6769, Sigma Chem Co, USA) in a buffer solution, pH 7.4, 100 μ L. The clotting time was measured in an automatic device (KC 10, Amelung, Germany).

- 5 The concentrations of the active thrombin inhibitor $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ (see International Patent Application WO 94/29336) in the rat plasma were estimated by the use of standard curves relating the thrombin time in the pooled citrated rat plasma to known concentrations of the aforementioned active thrombin inhibitor dissolved in saline.

10

Based on the estimated plasma concentrations of the active thrombin inhibitor $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ (which assumes that thrombin time prolongation is caused by the aforementioned compound) in the rat, the area under the curve after oral and/or parenteral administration of the

15 prodrug was calculated (AUCpd) using the trapezoidal rule and extrapolation of data to infinity.

The bioavailability of the active thrombin inhibitor $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ after oral or parenteral administration of the prodrug was

20 calculated as below:

$$[(\text{AUCpd}/\text{dose})/(\text{AUCactive,iv}/\text{dose})] \times 100$$

where AUCactive,iv represents the AUC obtained after intravenous

25 administration of $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ to conscious rats as described above.

Test CDetermination of thrombin time in urine *ex vivo*

5 The amount of the active thrombin inhibitor HO(O)C-CH₂(R)Cgl-Aze-Pab-H that was excreted in urine after oral or parenteral administration of the compounds of the invention, dissolved in ethanol:Solutol™:water (5:5:90), was estimated by determination of the thrombin time in urine *ex vivo* (assuming that thrombin time prolongation is caused by the aforementioned compound).

10

Conscious rats were placed in metabolism cages, allowing separate collection of urine and faeces, for 24 hours following oral administration of compounds of the invention. The thrombin time was determined on the collected urine as described below.

15

Pooled normal citrated human plasma (100μL) was incubated with the concentrated rat urine, or saline dilutions thereof, for one minute. Plasma coagulation was then initiated by the administration of human thrombin (T 6769, Sigma Chem Company) in buffer solution (pH 7.4; 100μL). The clotting time was measured in an automatic device (KC 10; Amelung).

20

The concentrations of the active thrombin inhibitor HO(O)C-CH₂(R)Cgl-Aze-Pab-H in the rat urine were estimated by the use of standard curves relating the thrombin time in the pooled normal citrated human plasma to known concentrations of the aforementioned active thrombin inhibitor dissolved in concentrated rat urine (or saline dilutions thereof). By multiplying the total rat urine production over the 24 hour period with the estimated mean concentration of the aforementioned active inhibitor in the urine, the amount of the active inhibitor excreted in the urine (AMOUNT_{pd}) could be calculated.

30

The bioavailability of the active thrombin inhibitor $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ after oral or parenteral administration of the prodrug was calculated as below:

$$[(\text{AMOUNT}_{\text{pd}}/\text{dose})/(\text{AMOUNT}_{\text{active,iv}}/\text{dose})] \times 100$$

where $\text{AMOUNT}_{\text{active,iv}}$ represents the amount excreted in the urine after intravenous administration of $\text{HO(O)C-CH}_2(\text{R})\text{Cgl-Aze-Pab-H}$ to conscious rats as described above.

10

Test D

Determination of $\text{HO(O)C-CH}_2-(\text{R})\text{Cgl-Aze-Pab-H}$ in urine by LC-MS

15

The amount of the active thrombin inhibitor $\text{HO(O)C-CH}_2-(\text{R})\text{Cgl-Aze-Pab-H}$ that was excreted in urine after oral or parenteral administration of the compounds of the invention, dissolved in ethanol:Solutol™:water (5:5:90), was measured by LC-MS analysis as described below.

20

The animal studies were performed as described in Method C above. Urine samples were collected and frozen at -20°C before they were analysed.

Urine samples were analysed for their content of $\text{HO(O)C-CH}_2-(\text{R})\text{Cgl-Aze-Pab-H}$ according to the following method:

25

Thawed urine samples were mixed and, if required, spinned in a centrifuge. Solid phase extraction tubes (Analytichem Bond Elut. No. 1210-2059) were activated with 1.0 mL of methanol and conditioned with 1.0 mL of acetonitrile:water (50:50), followed by 1.0 mL of 0.1 % formic acid. 50 μL of the working internal standard (20 $\mu\text{mol/L}$) was added to

30

each extraction tube. For urine standards, 50 μ L of standard solution was added. 200 μ L of a sample or, for urine standards, blank urine was added to each tube and thereafter pulled through *via* gravity or a gentle vacuum. Residual urine was washed out with 1.0 mL of ammonium acetate (2 mmol/L), before elution with 1.0 mL of acetonitrile:ammonium acetate (2 mmol/L) (35:65). The collected eluate was transferred to autosampler vials. 30 μ L of the extract was injected onto the LC column (Hypersil BDS-C18; 3 μ m; 75 mm x 4.0 mm i.d.; Hewlett-Packard No. 79926 03-354), eluted with ammonium acetate buffer (1.3 mmol/L) with 40% acetonitrile and 0.1 % formic acid at 0.75 mL/min. The effluent was split so that 30 μ L/min entered the electrospray ion source of a P-E Sciex API-3 mass spectrometer. HO(O)C-CH₂-(R)Cgl-Aze-Pab-H and HO(O)C-CH₂-(R)Cgl-Pro-Pab-H (internal standard) both have retention times near 1.5 minutes. Their molecular ions ((M+H)⁺) were monitored at m/z 430.2 and 444.2 respectively, at unit mass resolution. Urine standards at two levels, one being at the limit of quantification, were used for calibration based on peak area ratios of HO(O)C-CH₂-(R)Cgl-Aze-Pab-H over the internal standard. Linearity of the method was checked over the range 0.050-20 μ mol/L. The coefficient of variation was 1-2 % at 1-20 μ mol/L and 7 % at 0.50 μ mol/L. The limit of quantification was 0.050 μ mol/L.

By multiplying the total urine production over the 24 hour period by the measured concentration of HO(O)C-CH₂-(R)Cgl-Aze-Pab-H in the urine, the amount of the active inhibitor excreted in urine (AMOUNT_{pd}) could be calculated. The bioavailability of the active thrombin inhibitor was then calculated as described in Method C above.

The invention is illustrated by way of the following examples.

Examples

General Experimental Procedures.

- 5 Mass spectra were recorded on a Finnigan MAT TSQ 700 triple quadrupole mass spectrometer equipped with an electrospray interface.

The ^1H NMR and ^{13}C NMR measurements were performed on BRUKER ACP 300 and Varian UNITY plus 400 and 500 spectrometers, operating
 10 at ^1H frequencies of 300.13, 399.96 and 499.82 MHz respectively, and at ^{13}C frequencies of 75.46, 100.58 and 125.69 MHz respectively. Chemical shifts are reported in δ units.

Preparation of starting materials

- 15 Boc-(R)Cgl-Aze-Pab-H, Boc-(R)Cgl-Aze-Pab x HCl, H-(R)Aze-Pab-Z, H-(R)Aze-Pab-Z x HCl, Bn-OOCCH₂-(R)Cgl-Aze-Pab-Z, Boc-(R)Cgl-Aze-Pab-Z, Boc-(R)Cgl-Aze-OH and Pab-Z x HCl were prepared according to the methods described in International Patent
 20 Application WO 94/29336.

Example 1

EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂

- 25 (i) Boc-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂

To a solution of Boc-(R)Cgl-Aze-Pab-H (6.1 g; 13 mmol) in THF (125 mL) and 2M NaOH (70 mL; 140 mmol) at 0°C was added dropwise allyl chloroformate (1.7 g; 14 mmol). After stirring at 0°C for 1h, the reaction was mixture concentrated, water was added (100 mL) and the resulting
 30 aqueous phase was extracted with methylene chloride (3x100 mL). The

combined organic phases were concentrated to give 6.4 g of a crude product which was purified by flash chromatography using EtOAc:THF:Et₃N (68:29:3) as eluent. Concentration gave 5.8 g (81 %) of the subtitle compound as a white solid.

5

¹H NMR (500 MHz, CDCl₃): δ 8.19 (bt, 1H), 7.78 (d, 2H), 7.26 (d, 2H), 6.02-5.92 (m, 1H), 5.32 (d, J=17 Hz, 1H), 5.18 (d, J=10 Hz, 1H), 5.06 (d, J=7 Hz, 1H), 4.82 (bs, 1H), 4.61 (d, J=6 Hz, 2H), 4.58-4.48 (m, 1H), 4.38-4.27 (m, 2H), 4.14-4.03 (m, 1H), 3.77-3.68 (m, 1H), 2.60-0.90 (m, 24H).

10

¹³C NMR (125 MHz, CDCl₃) carbonyl and amidine signals: δ 172.70, 170.74, 168.02, 164.54, 155.98.

(ii) H-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂ x 2TFA

15

To a solution of Boc-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂ (2.03 g; 3.65 mmol; from step (i) above) in methylene chloride (15 mL) at 0°C was added TFA (15 mL). The reaction mixture was stirred at ambient temperature for 3 h followed by concentration to give the 2.8 g of the subtitle compound as a white solid.

20

¹H NMR (500 MHz, MeOH (d₄)): δ 7.80 (d, 2H), 7.57 (d, 2H), 6.02 (m, 1H), 5.45 (d, J=17 Hz, 1H), 5.33 (d, J=10 Hz, 1H), 5.91-4.80 (m, 3H), 4.56 (s, 2H), 4.38 (bq, J=8 Hz, 1H), 3.71 (d, J=7 Hz, 1H), 2.76-2.60 (m, 1H), 2.35-2.20 (m, 1H), 1.9-1.0 (m, 11H).

25

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂

A mixture of H-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂ x 2TFA (649 mg; 0.95 mmol; from step (ii) above), K₂CO₃ (656 mg, 4.8 mmol), water (0.1 mL), and THF (10 mL) was stirred at 40°C for 2 h followed by addition of ethyl bromoacetate (190 mg; 1.14 mmol) in THF (1 mL). After stirring

30

at 40°C for 4 h and at ambient temperature for 14 h the reaction mixture was filtered, concentrated, and purified by flash chromatography using EtOAc:THF:Et₃N (68:29:3) as eluent to give 244 mg (47%) of the title compound as a white solid.

5

¹H NMR (400 MHz, CDCl₃): δ 8.46 (bt, 1H), 7.81 (d, 2H), 7.35 (d, 2H), 6.08-5.94 (m, 1H), 5.35 (d, J=18 Hz, 1H), 5.23 (d, J=11 Hz, 1H), 4.93 (dd, J=6 and 9 Hz, 1H), 4.66 (d, 2H), 4.62-4.38 (AB part of an ABX-spectrum), 4.16-4.04 (m, 4 H), 3.20 (d, 2H), 2.86 (d, 1H), 2.64-2.45 (m, 2H), 2.0-1.0 (m 17H).

10

¹³C NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 175.33, 172.24, 170.72, 168.19, 164.35.

Example 2

15

*n*PrOOCCH₂-(*R*)Cgl-Aze-Pab-COOCH₂CH=CH₂

The title compound was prepared according to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-COOCH₂CH=CH₂ x 2TFA (503 mg; 0.74 mmol; see Example 1(ii) above) and *n*-propyl bromoacetate (160 mg, 0.88 mmol) to give 277 mg (68%) as a white solid.

20

¹H-NMR (400 MHz, CDCl₃): δ 8.48 (bt, 1H), 7.83 (d, 2H), 7.35 (d, 2H), 6.76 (broad, 1H), 6.02 (m, 1H), 5.37 (dd, 1H), 5.24 (dd, 1H), 4.94 (t, 1H), 4.67 (dd, 2H), 4.49 (AB part of an ABX-spectrum, 2H), 4.12 (m, 2H), 3.98 (t, 2H), 3.24 (AB-system, 2H), 2.87 (d, 1H), 2.52 (m, 2H), 1.99 (bd, 2H), 1.80-1.50 (m, 7H), 1.61 (q, 2H), 1.30-1.10 (m, 2H), 1.00 (qd, 2H), 0.90 (t, 3H).

25

¹³C-NMR (100 MHz, CDCl₃) amidine and carbonyl signals: δ 175.4, 172.3, 170.7, 167.9, 164.5

Example 3 $t\text{BuOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOCH}_2\text{CH=CH}_2$

The title compound was prepared according to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂ x 2TFA (285 mg; 0.42 mmol; see Example 1(ii) above) and *t*-butyl bromoacetate (96 mg; 0.50 mmol) to give 93 mg (39%) as a white solid.

¹H NMR (500 MHz, CDCl₃): δ 8.50 (bt, 1H), 7.81 (d, 2H), 7.36 (d, 2H), 6.07-5.97 (m, 1H), 5.36 (d, J=16 Hz, 1H), 5.22 (d, J=10 Hz, 1H), 4.93 (dd, J=9 and 6 Hz, 1H), 4.76 (d, J=6 Hz, 2H), 4.57-4.46 (m, 2H), 4.18-4.04 (m, 2H), 3.19-3.08 (AB-spectrum, J_{AB} = 20 Hz, 2H), 2.86 (d, J=8 Hz, 1H), 2.72-2.53 (m, 2H), 2.0-0.9 (m, 23H).

¹³C NMR (100 MHz, CDCl₃) amidine and carbonyl signals: δ 175.28, 171.53, 170.76, 167.81, 164.1.

Example 4 $\text{EtOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOEt}$ (i) Boc-(R)Cgl-Aze-Pab-COOEt

The sub-title compound was prepared according the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab-H (600 mg; 1.3 mmol) and ethyl chloroformate (150 mg; 1.4 mmol) yielding 240 mg (34%) as a white solid.

¹H-NMR (300 MHz, CDCl₃): δ 9.37 (bs, 1H), 8.16 (bs, 1H), 7.72 (d, 2H), 7.18 (d, 2H), 5.17 (d, 1H), 4.73 (t, 1H), 4.47 (dd, 1H), 4.27 (m, 2H), 4.06 (q, 2H), 3.66 (t, 1H), 2.48 (m, 1H), 2.37 (m, 1H), 1.4-1.8 (m, 7H), 1.22 (s, 9H), 1.3-0.8 (m, 7H).

¹³C-NMR (75MHz, CDCl₃) carbonyl and amidine signals: δ 172.6, 170.7, 167.9, 164.8, 156.0

(ii) H-(R)Cgl-Aze-Pab-COOEt x 2HCl

To a solution of Boc-(R)Cgl-Aze-Pab-COOEt (240 mg; 0.44 mmol; from step (i) above) in EtOAc (20 mL) was added hydrogen chloride at 0°C over 5 minutes. The reaction mixture was stirred at 0°C for 1 h followed by concentration to give 225 mg (100%) as a white solid.

¹H-NMR (300 MHz, D₂O): δ 7.85 (d, 2H), 7.61 (d, 2H), 4.98 (dd, 1H), 4.60 (s, 1H), 4.44 (p, 5H), 3.90 (d, 1H), 2.73 (m, 1H), 2.37 (m, 1H), 2.0-1.65 (m, 9H), 1.39 (t, 3H), 1.4-1.1 (m, 7H), 0.98 (m, 1H).

¹³C-NMR (75 MHz, D₂O) amidine and carbonyl signals: δ 172.7, 169.4, 166.8, 154.3.

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOEt

The title compound was prepared according to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOEt x 2HCl (160 mg; 0.31 mmol; from step (ii) above) and ethyl bromoacetate (52.5 mg; 0.31 mmol). Yield: 100 mg (61%) as a light yellow powder.

¹H-NMR (300 MHz, CDCl₃): δ 8.48 (bt, 1H), 7.81 (d, 2H), 7.38 (d, 2H), 4.51 (AB part of an ABX-spectrum, 2H), 4.21 (q, 2H), 4.15-4.05 (m, 4H), 3.21 (AB-spectrum, 2H), 2.86 (d, 1H), 2.68 (m, 1H), 2.53 (m, 1H), 1.96 (bd, 2H), 1.90-1.70 (m, 12H), 1.35 (t, 3H), 1.22 (t, 6H), 1.30-0.95 (m, 2H).

¹³C-NMR (75 MHz, CDCl₃) carbonyl and amidine signals: δ 175.5, 172.2, 170.7, 167.6, 164.9

Example 5EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Pr(i) Boc-(R)Cgl-Aze-Pab-COO-*n*Pr

- 5 The sub-title compound was prepared according to the procedure described in Example 1(i) above using Boc-(R)Cgl-Aze-Pab-H (6.0 g; 13 mmol;) and *n*-propyl chloroformate (1.57 mL; 14 mmol). Yield 5.4 g (76%).

¹H-NMR (400 MHz, CDCl₃): δ 8.25 (bt, 1H), 7.82 (d, 2H), 7.31 (d, 2H),
 10 5.09 (bd, 1H), 4.87 (dd, 1H), 4.58 (dd, 1H), 4.39 (dd, 2H), 4.14 (q, 1H), 4.10 (t, 2H), 3.79 (t, 1H), 2.54 (dm, 2H), 2.21 (s, 1H), 1.87-1.55 (m, 8H), 1.33 (s, 9H), 1.45-1.0 (m, 4H), 0.99 (t, 3H).

¹³C-NMR (100 MHz, CDCl₃) amidine and carbonyl signals: δ 172.7, 170.6, 167.8, 165.0, 155.9.

15

(ii) H-(R)Cgl-Aze-Pab-COO-*n*Pr x 2TFA

The sub-title compound was prepared according to the procedure described in Example 1(ii) using 2.1 g (3.7 mmol) of Boc-(R)Cgl-Aze-Pab-COO-*n*Pr (from step (i) above). Yield 3.7 g.

20

¹H-NMR (400 MHz, MeOH-d₄): δ 7.77 (d, 2H), 7.60 (d, 1H), 4.86 (dd, 1H), 4.56 (AB part of an ABX-spectrum, 2H), 4.33 (m, 4H), 3.72 (d, 1H), 3.30 (m, 1H), 2.68 (m, 1H), 2.28 (m, 1H), 1.9-1.7 (m, 9H), 1.4-1.1 (m, 6H), 1.02 (t, 3H).

25 ¹³C-NMR (100 MHz, MeOH-d₄) carbonyl and amidine signals: δ 172.7, 169.3, 168.0, 161.4.

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Pr

- The title compound was prepared according to the procedure described in
 30 Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-*n*Pr x 2TFA (472 mg; 0.69

mmol; from step (ii) above) and ethyl bromoacetate (138 mg; 0.83 mmol) to give 0.22 mg (58%) as a white solid.

¹H-NMR (400 MHz, CDCl₃): δ 8.46 (bt, 1H), 7.82 (d, 2H), 7.32 (d, 2H),
 5 4.92 (dd, 1H), 4.49 (AB part of an ABX-spectrum, 2H), 4.10 (m, 6H),
 3.23 (AB-spectrum, 2H), 2.80 (dm, 2H), 1.98 (bd, 2H), 1.74 (q, 2H),
 1.63 (dd, 2H), 1.52 (m, 1), 1.21 (t, 3H), 1.20-1.10 (m, 2H), 0.98 (t,
 3H).

¹³C-NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 175.3,
 10 172.2, 170.7, 167.6, 164.8.

Example 6

MeOOCCH₂-(R)Cgl-Aze-Pab-COO-nPr

The title compound was prepared according to the procedure described in
 15 Example 1(iii) above from H-(R)Cgl-Aze-Pab-COO-nPr x 2TFA (365 mg;
 0.53 mmol; see Example 5(ii) above) and methyl bromoacetate (98 mg;
 0.64 mmol) to give 114 mg (41%) as a white solid.

¹H-NMR (500 MHz, CDCl₃): δ 8.44 (bt, 1H), 7.82 (d, 2H), 7.32 (d,
 20 2H), 7.04 (broad, 1H), 4.92 (dd, 1H), 4.49 (AB part of an ABX
 spectrum), 4.12 (m, 2H), 4.10 (t, 2H), 3.63 (s, 3H), 3.24 (s, 2H), 2.87
 (d, 1H), 2.65 (m, 1H), 2.52 (m, 1H), 2.01 (broad, 1H), 1.96 (bd, 2H),
 1.75 (q, 4H), 1.63 (bdd, 1H), 1.53 (m, 1H), 1.3-1.1 (m, 5H), 0.99 (t,
 3H).

¹³C-NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 175.3,
 25 172.5, 170.7, 167.7, 165.0.

Example 7EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe(i) Boc-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe

- 5 The sub-title compound was prepared according to the procedure described in Example 1(i) above using Boc-(R)Cgl-Aze-Pab-H (6.0 g; 13 mmol) and 2-methoxyethyl chloroformate (1.94 g; 14 mmol). Yield 3.9 g (52%).

10 ¹H-NMR (400 MHz, CDCl₃): δ 8.24 (bt, 1H), 7.83 (d, 2H), 7.31 (d, 2H), 5.08 (bd, 1H), 4.87 (dd, 1H), 4.58 (dd, 1H), 4.39 (dd, 2H), 4.30 (t, 2H), 4.15 (m, 1H), 3.79 (bt, 1H), 3.68 (t, 2H), 3.40 (s, 3H), 2.65-2.45 (m, 2H), 2.20 (broad, 1H), 1.9-1.55 (m, 6H), 1.34 (s, 9H), 1.3-0.95 (m, 6H).
¹³C-NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 172.7, 170.7, 167.8, 164.6, 155.9.

15

(ii) H-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe x 2TFA

The sub-title compound was prepared according to the procedure described in Example 1(ii) above using 1.71 g of Boc-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe (from step (i) above). Yield 1.89 g (88%).

20

¹H-NMR (400 MHz, MeOH-d₄): δ 7.77 (d, 2H), 7.59 (d, 2H), 4.85 (dd, 1H), 4.56 (d, 2H), 4.49 (m, 2H), 4.37 (m, 1H), 4.28 (m, 1H), 3.70 (m, 3H), 3.37 (s, 3H), 2.68 (m, 1H), 2.28 (m, 1H), 1.9-1.7 (m, 7H), 1.4-1.1 (m, 6H).

25

¹³C-NMR (100 MHz, MeOH-d₄) carbonyl and amidine signals: δ 172.7, 169.3, 168.0, 154.6.

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe

The title compound was prepared according to the procedure described in
 30 Example 1(iii) above from H-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe x 2TFA

(487 mg; 0.69 mmol; from step (ii) above) and ethyl bromoacetate (138 mg; 0.83 mmol) to give a crude product which was purified by flash chromatography using THF:methylene chloride (3:1) as eluent. The yield was 0.13 mg (34 %) as a white solid.

5

¹H-NMR (400 MHz, CDCl₃): δ 8.46 (bt, 1H), 7.83 (d, 2H), 7.32 (d, 2H), 7.21 (broad, 1H), 4.92 (dd, 1H), 4.49 (AB part of an ABX spectrum, 2H), 4.30 (t, 2H), 4.12 (q, 2H), 4.07 (q, 2H), 3.68 (t, 1H), 3.40 (s, 3H), 3.24 (s, 2H), 2.62 (m, 1H), 2.52 (m, 1H), 2.07 (broad, 1H), 1.97 (bd, 1H), 1.8-1.5 (m, 5H), 1.3-1.1 (m, 6H), 1.05-0.95 (m, 2H).

10

¹³C-NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 175.3, 172.2, 170.7, 167.8, 164.6.

Example 8

15 MeOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe

The title compound was prepared according to the method described in Example 1(iii) above from H-(R)Cgl-Aze-Pab-COOCH₂CH₂OMe x 2TFA (490 mg; 0.7 mmol; see Example 7(ii) above) and methyl bromoacetate (128 mg; 0.84 mmol) to give a crude product which was purified by flash chromatography using THF:methylene chloride (3:1) as eluent. The yield was 155 mg (41 %) as a white solid.

20

¹H-NMR (400 MHz, CDCl₃): δ 8.44 (t, 1H), 7.83 (d, 2H), 7.31 (d, 2H), 4.92 (dd, 1H), 4.49 (AB part of an ABX spectrum, 2H), 4.30 (t, 2H), 4.13 (m, 2H), 3.68 (t, 2H), 3.63 (s, 3H), 3.39 (s, 3H), 3.25 (s, 2H), 2.87 (d, 1H), 2.62 (m, 1H), 2.52 (m, 1H), 1.96 (bd, 1H), 1.8-1.5 (m, 6H), 1.3-1.1 (m, 5H), 1.00 (q, 2H).

25

¹³C-NMR (100 MHz, CDCl₃) carbonyl and amidine signals: δ 175.2, 172.6, 170.7, 167.8, 164.5.

30

Example 9EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Bu(i) Boc-(R)Cgl-Aze-Pab-COO-*n*Bu

5 The sub-title compound was prepared according to the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab-H (1.01 g; 2.1 mmol) and *n*-butyl chloroformate (0.32 g; 2.4 mmol). After stirring at ambient temperature for 1.5 h the reaction mixture was concentrated and extracted with three portions of methylene chloride. The combined organic phase
 10 was then washed with water, dried over Na₂SO₄, and concentrated to give 1.0 g (83 %) of the sub-title compound as a white solid.

¹H-NMR (300 MHz, CDCl₃): δ 9.81-9.31 (bs, 1H), 8.36-8.20 (m, 1H), 7.35 (d, 2H), 7.84 (d, 2H), 6.78-6.43 (bs, 1H), 5.05-4.82 (m, 2H), 4.69-
 15 4.15 (m, 3H), 4.15-4.08 (m, 3H), 3.86-3.70 (m, 1H), 2.68-2.42 (m, 2H), 1.92-0.88 (m, 25H).

¹³C-NMR (125 MHz, CDCl₃) amidine and carbonyl signals: δ 172.5, 170.7, 167.9, 164.9, 156.0.

FAB-MS: (m+1)=572 (m/z)

20

(ii) H-(R)Cgl-Aze-Pab-COO-*n*Bu x 2HCl

The sub-title compound was prepared according to the procedure described in Example 4(ii) from Boc-(R)Cgl-Aze-Pab-COO-*n*Bu (2.5 g; 4.4 mmol; from step (i) above) to give 2.4 g (100%) as a white solid.

25

¹H-NMR (300 MHz, MeOH-d₄): δ 7.78-7.60 (m, 2H), 4.66 -4.49 (m, 2H), 0.98 (t, 2H), 4.49-4.35 (m, 3H), 4.35-4.22 (m, 1H), 3.75 (d, 1H), 1.92-1.67 (m, 8H), 1.56-1.07 (m, 8H). The signal of one of the protons is partially obscured by the CD₃OH-signal

30 ¹³C-NMR (100 MHz, MeOH-d₄) amidine and carbonyl signals: δ 172.7,

169.3, 167.9, 154.7

MS (m+1)=472 (m/z)

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COO-nBu

- 5 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-nBu x 2HCl (400 mg; 0.74 mmol) and ethyl bromoacetate (147 mg; 0.88 mmol). The product was purified by flash chromatography using methylene chloride and EtOH gradient 0.1 % = > 12.8 % as eluent to give 290 mg (70%) as a white solid.

10

¹H-NMR (300MHz, CDCl₃): δ 9.70-9.36 (bs, 1H), 8.47 (t, 1H), 7.81 (d, 2H), 7.32 (d, 2H), 7.07-6.73 (bs, 1H), 4.97-4.87 (dd, 1H), 4.62-4.35 (m, 2H), 4.20-3.98 (m, 6H), 3.27-3.12 (m, 2H), 2.84 (s, 1H), 2.70-2.40 (m, 2H), 2.03-0.85 (m, 22H)

15

¹³C-NMR (75MHz, CDCl₃) amidine and carbonyl signals: δ 175.3, 172.3, 170.8, 167.9, 165.0

FAB-MS: (m+1)=558 (m/z)

Example 10

20

PrIC(O)CH₂CH₂CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z

(i) PrIC(O)CH₂CH₂CH₂OH

- A mixture of γ-butyrolactone (4.0 g; 46.5 mmol) and pyrrolidine (6.6 g; 92.8 mmol) was stirred at room temperature for 2.5 h. The product was concentrated in vacuum to give 14.5 g (100%) of the product as a yellow oil.

25

¹H-NMR (300MHz, MeOH-d₄): δ 3.58 (t, 2H), 3.50 (t, 2H), 3.40 (t, 2H), 2.42 (t, 2H), 2.06-1.75 (m, 6H)

(ii) PrIc(O)CH₂CH₂CH₂OOCCH₂Br

To a mixture of PrIc(O)CH₂CH₂CH₂OH (7.2 g; 45.8 mmol; from step (i) above) and DMAP (5.6 g; 45.8 mmol) in methylene chloride at 0°C was added dropwise bromoacetyl bromide (4.0 mL; 45.8 mmol). After stirring at room temperature for 1.5 h another portion of bromoacetyl bromide (1.0 mL, 11.4 mmol) and DMAP (1.4 g, 11.4 mmol) was added and reaction was refluxed for 1.5 h. Water was added and the methylene chloride was extracted 3 times. The organic phase was dried with Na₂SO₄ and concentrated to give 10.3 g (81%) of the product as a yellow oil.

¹H-NMR (400 MHz, CDCl₃): δ 4.15 (t, 2H), 3.75 (s, 2H), 3.40-3.31 (m, 4H), 2.30 (t, 2H), 1.98-1.83 (m, 4H), 1.81 -1.73 (m, 2H)

(iii) PrIc(O)CH₂CH₂CH₂OOCCH₂-(R)Cgl-Aze-Pab(Z)

The title compound was prepared according to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (6 g; 10.4 mmol) and PrIc(O)CH₂CH₂CH₂OOCCH₂Br (3.5 g; 12.4 mmol; from step (ii) above). The crude product was purified by flash chromatography using heptane:EtOAc:isopropanol (1:2:2) as eluent to give 4.2 g which was then purified by using preparative RPLC using 44% acetonitrile in 0.1M NH₄OAc as eluent to give 2.64 g (36%) of the product as a white solid.

¹H-NMR (500 MHz, CDCl₃): δ 9.80- 9.22 (b s, 1H), 8.36 (t, 1H), 7.96 -7.58 (m, 3H), 7.45 (d, 2H), 7.37 -7.22 (m, 5H), 5.20 (s, 2H), 4.95 -4.88 (dd, 1H), 4.72 -4.29 (m, 2H), 4.15 -4.04 (m, 2H), 4.04 -3.88 (m, 2H), 3.40 (t, 2H), 3.34 (t, 2H), 3.28 -3.17 (m, 2H), 2.85 (d, 1H), 2.67 -2.48 (m, 1H), 2.23 (t, 2H), 2.14 -0.93 (m, 18H).

¹³C-NMR (125 MHz, CDCl₃) amidine and carbonyl signals: δ 175.3, 172.4, 170.9, 170.4, 168.2, 164.6

FAB-MS: (m+1)=703 (m/z)

Example 11ChNHC(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z(i) ChNHC(O)CH₂OH

- 5 A mixture of cyclohexylamine (9.9 g; 99.8 mmol) and 2,5-dioxo-1,4-dioxane (3.0 g, 25.9 mmol) was stirred at 100°C for 2.5 h. The product was concentrated to give 8.1 g (100%) of the product as a brown solid.

10 ¹H-NMR (500 MHz, MeOH-d₄): δ 3.92 (s, 2H), 3.75 -3.65 (m, 1H), 1.90 -1.58 (m, 5H), 1.43 -1.07 (m, 5H). The signal of two of the protons are obscured by the CD₃OH-signal.

¹³C-NMR (125 MHz, MeOH-d₄) amidine and carbonyl signals: δ 174.0, 62.5, 33.7, 26.5, 26.1, 26.0 The signal of one of the carbons is obscured by the CD₃OD-signal.

15

(ii) ChNHC(O)CH₂OOCCH₂Br

To a mixture of ChNHC(O)CH₂OH (8.0 g; 50.9 mmol; from step (i) above) and DMAP (6.2 g; 50.9 mmol) in methylene chloride (80 mL) at 0°C was added dropwise bromoacetyl bromide (4.0 mL; 45.8 mmol).

- 20 After stirring at room temperature for 1.5 h further portions of bromoacetyl bromide (1.0 mL, 11.4 mmol) and DMAP (1.4 g, 11.4 mmol) were added and the reaction mixture was refluxed for 1.5 h. Water was added and the aqueous phase was extracted with three portions of methylene chloride. The organic phase was washed with water, dried with
25 Na₂SO₄, and concentrated to give 10.3 g (73%) of the product as a brown solid.

30 ¹H NMR (400 MHz, CDCl₃): δ 6.12-6.00 (bs, 1H), 4.62 (s, 2H), 3.90 (s, 2H), 3.84-3.76 (m, 1H), 1.95-1.86 (m, 2H), 1.75-1.65 (m, 2H), 1.65 - 1.56 (m, 1H), 1.43-1.29 (m, 2H), 1.24-1.10 (m, 3H).

(iii) ChNHC(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) with starting from H-(R)Cgl-Aze-Pab-Z (6 g; 10.4 mmol) and ChNHC(O)CH₂OOCCH₂Br (3.5 g; 12.4 mmol; from step (ii) above). The crude product was purified by flash chromatography using heptane:EtOAc:isopropanol (5:2:2) as eluent followed by concentration and then by preparative RPLC using 50% acetonitrile in 0.1M NH₄OAc as eluent. Concentration and freeze drying gave 2.6 g (36%) of the product as a white solid.

10

¹H-NMR (500 MHz, CDCl₃): δ 9.78-9.25 (bs, 1H), 7.90 (t, 1H), 7.78 (d, 2H), 7.44 (d, 2H), 7.38-7.24 (m, 5H), 6.66 (t, 1H), 5.20 (s, 2H), 4.90-4.83 (dd, 1H), 4.60-4.45 (m, 2H), 4.18-3.93 (m, 4H), 3.73-3.62 (m, 1H), (d, 1H), 3.23, 3.44 (AB, 2H), 2.87, 2.65-2.08 (m, 3H), 1.98-0.93 (m, 22H)

15

¹³C-NMR (125 MHz, CDCl₃) amidine and carbonyl signals: δ 175.1, 171.7, 170.7, 168.8, 166.1, 164.4

FAB-MS: (m+1)=703 (m/z)

20 Example 12(nPr)₂NC(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OCCC(CH₃)₃(i) (nPr)₂NC(O)CH₂OH

A mixture of 2,5-dioxo-1,4-dioxane (2.02 g; 17.4 mmol) and di-*n*-propylamine (5 ml; 36.5 mmol) was heated at 50°C for 1 h and at 90°C for 66 h. Toluene was added and subsequently removed in vacuo with excess di-*n*-propylamine. The residue was purified by flash chromatography using 10% methanol in methylene chloride as eluent to give 4.18 g (66%) of the desired compound.

30

^1H NMR (300 MHz, CDCl_3): δ 4.1 (d, 2 H), 3.65 (t, 1 H), 3.25-3.35 (m, 2 H), 2.9-3.0 (m, 2 H), 1.45-1.6 (m, 4 H), 0.8-0.95 (m, 6 H)

(ii) $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OOCCH}_2\text{Br}$

- 5 A mixture of $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OH}$ (0.743 g; 4.7 mmol; from step (i) above), DCC (0.951 g, 4.6 mmol), and bromoacetic acid (0.704 g; 5.1 mmol) in methylene chloride (15 ml) was stirred at room temperature for 1.5 h. The precipitate was removed by filtration and the solvent was removed from the filtrate in vacuo. Kugelrohr distillation of the residue
10 gave 0.66 g (50%) of the desired compound.

^1H NMR (300 MHz, CDCl_3): δ 4.8 (s, 2 H), 4.0 (s, 2 H), 3.2-3.3 (m, 2 H), 3.05-3.15 (m, 2 H), 1.5-1.7 (m, 4 H), 0.8-1.0 (dt, 6 H)

15 (iii) Pivaloyloxymethyl 4-nitrophenyl carbonate

- A mixture of silver pivalate (7.5 g; 25 mmol) and iodomethyl 4-nitrophenyl carbonate (Alexander *et al*, J. Med. Chem. (1988) 31, 318; 7.99 g; 25 mmol) was refluxed in benzene (50 ml) for 2 h. The benzene was removed in vacuo and the residue was dissolved in toluene. Filtration
20 through hyflo and purification by flash chromatography using toluene as eluent afforded 4.00 g (54%) of the sub-title compound.

^1H NMR (300 MHz; CDCl_3): δ 8.25 (d, 2 H), 7.40 (d, 2 H), 5.85 (s, 2 H), 1.2 (s, 2 H)

- 25 ^{13}C NMR (75 MHz, CDCl_3) amidine and carbonyl signals: δ 176.77, 155.06

(iv) Boc-(R)Cgl-Aze-Pab-COOCH₂OCCC(CH₃)₃

- A solution of pivaloyloxymethyl 4-nitrophenyl carbonate (1.18 g; 4 mmol;
30 from step (iii) above) in methylene chloride (20 ml) was added at room

temperature to a solution of Boc-(*R*)Cgl-Aze-Pab-H (1.88g; 4 mmol) and triethylamine (0.66 ml; 4.75 mmol) in methylene chloride (20 ml). After 1 h the methylene chloride was replaced by EtOAc and the mixture was purified by flash chromatography using EtOAc as eluent to give 1.27 g
5 (50%) of sub-title compound.

¹H NMR (300 MHz, CDCl₃): δ 9.5 (bs, 1 H), 8.25 (t, 1 H), 7.8 (d, 2 H), 7.3 (d, 2 H), 7.0 (bs, 1 H), 5.0-4.8 (m, 2 H), 4.65-4.5 (m, 1 H), 4.5-4.3 (m, 2 H), 4.2-4.05 (m, 1 H), 3.75 (t, 1 H), 2.7-2.4 (m, 2 H), 1.9 -1.45
10 (m, 5 H), 1.45 - 0.8 (m, 24 H)

(v) H-(*R*)Cgl-Aze-Pab-COOCH₂OOCC(CH₃)₃

Boc-(*R*)Cgl-Aze-Pab-COOCH₂OOCC(CH₃)₃ (327 mg; 0.52 mmol; from step (iv) above) was dissolved in a mixture of methylene chloride (5 ml) and TFA (1.2 ml). After 2 h the reaction mixture was concentrated in
15 vacuo, acetonitrile was added, and the solvent was again removed in vacuo to give crude sub-title product which was used without further purification in the next step.

20 (vi) (*n*Pr)₂NC(O)CH₂OOCCH₂-(*R*)Cgl-Aze-Pab-COOCH₂OOCC(CH₃)₃

The residue from step (v) above was mixed with (*n*Pr)₂NC(O)CH₂OOCCH₂Br (150 mg; 0.53 mmol; from step (ii) above) and K₂CO₃ (480 mg; 3.5 mmol) in THF (5 ml) and heated for 3 h at 40°C. The reaction mixture was filtered and concentrated to a crude
25 product which was purified by preparative RPLC to give 78 mg (21%) of the title compound.

¹H NMR (300 MHz, CDCl₃): δ 9.3-9.6 (bs, 1 H), 8.5 (m, 1 H), 7.95-8.15 (bs, 1 H), 7.85-7.95 (d, 2 H), 7.2-7.3 (d, 2 H), 5.8 (s, 2 H), 4.8-4.9
30 (dd, 1 H), 4.5-4.7 (m, 3 H), 4.0-4.4 (m, 3 H), 2.8-3.4 (m, 5 H), 2.2-2.7

(m, 3 H), 1.75-1.3 (m, 9 H), 1.3-1.0 (m, 14 H), 1.0-0.7 (m, 7 H).

^{13}C NMR (75 MHz, CDCl_3) amidine and carbonyl signals: δ 177.24, 175.30, 171.85, 170.79, 168.78, 165.82, 163.14.

5 Example 13

$\text{EtOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOCH}_2\text{OOCCH(CH}_3)_3$

The title compound was prepared analogously to the procedure described in Example 12(vi) above from crude Boc-(R)Cgl-Aze-Pab-COOCH₂OOCCH(CH₃)₃ (0.41 g; 0.65 mmol; see Example 12(iv) above) using acetonitrile (10 ml) as solvent. After stirring over night at room temperature, the solvent was removed *in vacuo* and the residue was partitioned between EtOAc and water. The aqueous phase was extracted three times with EtOAc and the combined organic phases were dried (Na₂SO₄) and the solvent was removed *in vacuo*. The residue was subjected to flash chromatography using methylene chloride/methanol as eluent. Freeze drying from glacial acetic acid gave 84 mg (21%) of the title compound.

^1H NMR (300 MHz, CDCl_3): δ 9.9 (bs, 1 H), 8.5 (t, 1 H), 7.35 (d, 2 H), 5.85 (s, 2 H), 5.90 (dd, 2 H), 4.6-4.35 (m, 2 H), 4.15-4.0 (m, 4), 3.2 (s, 2 H), 2.85 (d, 1 H), 2.7-2.45 (m, 2), 2.0-1.9 (m, 2 H), 1.8-1.45 (m, 5 H), 1.3-0.9 (m, 18 H).

^{13}C NMR (75 MHz, CDCl_3) amidine and carbonyl signals: δ 177.23, 175.48, 172.29, 170.80, 168.85, 163.14.

25

Example 14

$\text{EtOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOCH(CH}_3\text{)OOCCH}_3$

(i) Boc-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCH₃

30 A solution of Boc-(R)Cgl-Aze-Pab-H (6.38 g; 13.5 mmol), 1-acetoxyethyl

4-nitrophenyl carbonate (Alexander *et al*, J. Med. Chem. (1988) 31, 318) (3.05 g; 12 mmol), and triethylamine (1.95 ml; 14 mmol) in methylene chloride (40 ml) was stirred at room temperature for 16 h followed by addition of EtOAc. The resulting solution was slightly concentrated and washed with aqueous Na₂CO₃ (10%), concentrated to a crude product, which was purified by flash chromatography using EtOAc as eluent, to give 5.59 g (77%) of the sub-title compound.

¹H NMR (300 MHz, CDCl₃): δ 9.5 (bs, 1 H), 8.25 (t, 1 H), 7.85 (d, 2 H), 7.35 (d, 2 H), 6.95 (q, 1 H), 6.7 (bs, 1 H), 5.0-4.85 (m, 2 H), 4.65-4.5 (m, 1 H), 4.5-4.25 (m, 2 H), 4.2-4.05 (m, 1 H) 3.75 (t, 1 H), 2.65-2.45 (m, 2 H) 2.05 (s, 3 H), 1.9-1.45 (m, 11 H), 1.45-0.8 (m, 12 H).
¹³C NMR (75 MHz, CDCl₃) amidine and carbonyl signals: δ 172.61, 170.80, 169.54, 168.91, 162.50, 156.02.

(ii) H-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCH₃

The crude sub-title compound was prepared according to the procedure described in Example 12(v) above from Boc-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCH₃ (2.21 g; 3.68 mmol; from step (i) above).

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCH₃

The crude H-(R)Cgl-Aze-Pab-COOCH(CH₃)OOCCH₃ from step (ii) above was dissolved in methylene chloride (150 mL). The mixture was washed with a 10% Na₂CO₃ solution and the organic phase was dried with K₂CO₃ and filtered. To the resulting solution as added K₂CO₃ (756 mg, 5.5 mmol) and ethyl (O-trifluoromethanesulphonyl)-glycolate (790 mg; 3.3 mmol) in methylene chloride (5 ml). The reaction mixture was stirred for 5-10 minutes at room temperature and then concentrated in vacuo. The residue was dissolved in EtOAc and the resulting mixture was filtered through celite. The filtrate was subjected to flash chromatography using

EtOAc as eluent followed by HPLC to give 475 mg (22%) of the title compound.

¹H NMR (300 MHz, CDCl₃): δ 9.5 (bs, 1 H), 8.3 (t, 1 H), 7.7 (d, 2 H),
 5 7.2 (d, 2 H), 6.85 (q, 1 H), 4.8 (t, 1 H), 4.45-4.25 (m, 2 H), 4.1-3.85
 (m, 4 H), 3.1 (s, 2 H), 2.75 (s, 1 H), 2.5-2.3 (m, 2 H), 1.95 (s, 3 H),
 1.9-1.8 (m, 1 H), 1.7-1.25 (m, 8 H), 1.25-1.75 (m, 8 H).

¹³C NMR (75.5 MHz, CDCl₃) amidine and carbonyl signals: δ 175.26,
 172.34, 170.81, 169.49, 168.80, 162.43.

10

Example 15

MeOOCCH₂-(R)Cgl-Aze-Pab-OOCPh

(i) Boc-(R)Cgl-Aze-Pab-OOCPh

15 To a solution of Boc-(R)Cgl-Aze-Pab-H (1.0 g; 2.1 mmol) and Na₂HPO₄
 (18.7 g; 105 mmol) in THF (45 mL) at 20° C was added dropwise
 dibenzoyl peroxide (556 mg; 2.3 mmol) dissolved in THF (10 mL) over
 45 minutes. After stirring at 20°C for 24 h, the reaction mixture was
 concentrated and the resulting crude product was subjected to preparative
 20 RPLC. This gave 124 mg (10%) of the sub-title compound as a white
 solid.

¹H-NMR (500 MHz, CDCl₃): δ 8.26 (m, 1H), 8.09 (m, 2H), 7.72 (m,
 2H), 7.59 (m, 1H), 7.48 (m, 2H), 7.36 (d, 2H), 5.13 (s, 2H), 4.87-4.98
 25 (m, 2H), 4.54-4.61 (m, 1H), 4.33-4.47 (m, 2H), 4.13-4.19 (m, 1H), 3.81
 (t, 1H), 2.53-2.63 (m, 2H), 1.73-1.86 (m, 3H), 1.66-1.72 (m, 2H), 1.36
 (s, 9H), 0.968-1.28 (m, 6H).

¹³C-NMR (100 MHz, CDCl₃) amidine and carbonyl signals: δ 172.7,
 170.6, 163.9, 157.0, 155.9.

30 LC-MS: m/z 592 (M+H⁺); m/z 614 (M+Na⁺).

(ii) H-(R)Cgl-Aze-Pab-OOCPh

To a solution of Boc-(R)Cgl-Aze-Pab-OOCPh (600 mg; 1.01 mmol; from step (i) above) in methylene chloride (18 mL) was added TFA (6 mL) at 20°C. After stirring for 14 h, the reaction mixture was concentrated and the resulting crude product was partitioned between EtOAc:0.1 M NaOH. The phases were separated and the organic layer was dried (Na₂SO₄) and evaporated. Yield: 480 mg (96%) as a white solid.

¹H-NMR (400 MHz, MeOH-d₄): δ 8.18 (m, 2H), 7.77 (m, 2H), 7.64 (m, 1H), 7.52 (m, 2H), 7.43 (d, 2H), 4.75-4.81 (m, 1H), 4.50 (s, 2H), 4.18-4.34 (m, 2H), 3.12 (d, 1H), 2.57-2.68 (m, 1H), 2.23-2.33 (m, 1H), 1.88-1.96 (m, 1H), 1.73-1.84 (m, 2H), 1.59-1.71 (m, 2H), 1.45-1.57 (m, 1H), 0.80-1.34 (m, 5H)

LC-MS: m/z 492 (M+H⁺); m/z 514 (M+Na⁺)

(iii) MeOOCCH₂-(R)Cgl-Aze-Pab-OOCPh

To a solution of H-(R)Cgl-Aze-Pab-OOCPh (480 mg; 0.97 mmol; from step (ii) above), K₂CO₃ (270 mg; 2 mmol) in acetonitrile (5 mL) at 20°C was added methyl bromoacetate (177 mg; 1.16 mmol). The reaction was stirred at 20°C for 14 h. The reaction mixture was filtered and concentrated to give a crude product which was purified by preparative RPLC to give 269 mg (49%) of the title compound as a white solid.

¹H-NMR (500 MHz, CDCl₃): δ 8.43 (m, 1H, NH), 8.09 (m, 2H), 7.69 (m, 2H), 7.59 (m, 1H), 7.47 (m, 2H), 7.34 (m, 2H), 5.27 (s, 2H), 4.93 (dd, 1H), 4.59 (dd, 1H), 4.40 (dd, 1H), 4.12 (m, 2H), 3.65 (s, 3H), 2.87 (d, 1H), 2.72-2.63 (m, 1H), 2.55-2.48 (m, 1H), 1.96 (m, 1H), 1.74 (m, 2H), 1.67 (d, 1H), 1.59 (d, 1H), 1.56-1.50 (m, 1H), 1.29-1.08 (m, 4H), 1.04-0.94 (m, 1H)

¹³C-NMR (100 MHz, CDCl₃) amidine and carbonyl signals: δ 175.1,

172.5, 170.6, 164.0, 157.1

LC-MS: m/z 564 ($M+H^+$)

Example 16

5 MeOOCCH₂-(R)Cgl-Aze-Pab-OH

To a solution of MeOOCCH₂-(R)Cgl-Aze-Pab-OC(O)Ph (260 mg; 0.46 mmol; see Example 15(iii) above) in THF (4.6 mL) was added KOMe (1.6 mL; 0.29 M; 0.46 mmol) at 20°C. After 15 minutes of stirring the mixture was concentrated and subjected to preparative RPLC. This gave
10 109 mg (52%) of the title compound as a white solid.

¹H-NMR (500 MHz, MeOH-d₄): δ 7.59 (d, 2H), 7.34 (d, 2H), 4.83 (s, 2H), 4.82-4.76 (m, 1H), 4.48 (d, 1H), 4.33 (d, 1H), 4.15-4.30 (m, 2H), 3.64 (s, 3H), 3.04 (d, 1H), 2.57 (m, 1H), 2.26 (m, 1H), 1.95 (m, 1H),
15 1.75 (m, 2H), 1.58-1.70 (m, 2H), 1.53 (m, 1H), 1.31-1.10 (m, 4H), 1.04 (m, 1H)

¹³C-NMR (100 MHz, MeOH-d₄): amidine and carbonyl signals: δ 175.9, 174.3, 172.7, 155.2

LC-MS: m/z 460 ($M+H^+$), m/z 482 ($M+Na^+$)

20

Example 17

EtOOCCH₂-(R)Cgl-Aze-Pab-OH

To a solution of EtOOCCH₂-(R)Cgl-Aze-Pab-C(O)OCH(CH₃)OOCCH₃ (184 mg; 0.31 mmol; see Example 14(iii) above), hydroxylamine hydrochloride (120mg; 1.72 mmol) and triethylamine (0.8 mL; 5.7 mmol) in EtOH (95%; 4.0 mL) was added, and the mixture stirred at room temperature for 4 days. The reaction mixture was concentrated and the crude product subjected to preparative RPLC. This gave 85mg (58%) of the title compound.

30

$^1\text{H-NMR}$ (300 MHz, CD_3OD): δ 7.6 (d, 2H), 7.35 (d, 2H), 4.75–4.85 (m, 1H), 4.4–4.55 (m, 2H), 4.0–4.35 (m, 4H), 3.35 (d, 2H), 3.05 (d, 1H), 2.5–2.65 (m, 1H), 2.2–2.35 (m, 1H), 1.9–2.05 (m, 1H), 1.4–1.85 (m, 5H), 0.85–1.35 (m, 8H)

5 $^{13}\text{C-NMR}$ (75.5 MHz, CD_3OD): amidine and carbonyl signals: δ 175.97, 173.91, 172.72, 155.23

LC-MS: $(m+1)=474$ (m/z)

Example 18

10 $\text{BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-OH}$

To a solution of hydroxylamine hydrochloride (320mg; 4.59mmol) and triethylamine (1.7ml; 12.24mmol) in EtOH, $\text{BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$ (1.0g; 1.52mmol) was added. The reaction mixture was stirred at room temperature for 40 hours and then concentrated. The crude product was
15 purified by preparative RPLC using 50% acetonitrile in 0.1M NH_4OAc as eluent to give 0.34g (42%) of the title compound.

LC-MS: $(m+1)=536$ (m/z)

20 Example 19

$n\text{PrOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from $\text{H-(R)Cgl-Aze-Pab-Z} \times 2\text{HCl}$ (700mg; 1.2mmol) and *n*-propyl bromoacetate (268mg; 1.45mmol). Yield 259mg (35%).

25

FAB-MS: $(m+1)=606$ (m/z)

Example 20

$n\text{PrOOCCH}_2\text{-(R)Cgl-Aze-Pab-OH}$

30 The title compound was prepared analogously to the procedure described

in Example 18 from $n\text{PrOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$ (182mg; 0.3 mmol; see Example 19 above). The crude product was purified by preparative RPLC using 40% acetonitrile in 0.1M NH_4OAc as eluent to give 74mg (51%) of the desired compound.

5

LC-MS: $(m+1)=488$ (m/z)

Example 21

$i\text{PrOOCCH}_2\text{-(R)Cgl-Aze-Pab-OH}$

10 The title compound was prepared analogously to the procedure described in Example 18 from $i\text{PrOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$ (590mg; 0.7mmol; see Example 39 below). Yield 110 mg (32%)

LC-MS: $(m+1)=488$ (m/z)

15

Example 22

$t\text{BuOOCCH}_2\text{-(R)Cgl-Aze-Pab-OH}$

The title compound was prepared analogously to the procedure described in Example 18 from $t\text{BuOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$ (738mg; 1.2mmol; see Example 37 below). Yield 290mg (48%).

20

LC-MS: $(m+1)=502$ (m/z)

Example 23

25 $(n\text{Pr})_2\text{NCOCH}_2\text{OOCCH}_2\text{-(R)Cgl-Aze-Pab(OH)}$

(i) $\text{HOOCCH}_2\text{-(R)Cgl(Boc)-Aze-Pab-O-Boc}$

A solution of $\text{HOOCCH}_2\text{-(R)Cgl-Aze-Pab-OH}$ (670mg; 1.5mmol; see Example 28 below), $(\text{Boc})_2\text{O}$ (654mg; 3mmol), and DMAP (92mg; 0.75mmol) in THF:water (10:1) was stirred at room temperature for 2 h.

30

The reaction mixture was concentrated and purified by preparative RPLC. Freeze drying yielded 112mg (12%) of the sub-title compound as a white solid.

5 LC-MS:(m-1)=643 (m/z)

(ii) (nPr)₂NCOCH₂OOCCH₂-(R)Cgl(Boc)-Aze-Pab-O-Boc

A solution of HOOCH₂-(R)Cgl(Boc)-Aze-Pab-O-Boc (100mg; 0.15mmol; from step (i) above), (nPr)₂NCOCH₂OH (27mg; 0.17mmol; see Example 12(i) above), EDC (40mg; 0.21mmol) and DMAP (10mg; 0.075mmol) in acetonitrile (5mL) was stirred at room temperature for 4 days. The reaction mixture was concentrated, purified by preparative RPLC and freeze dried to give 21mg (18%) of the sub-title compound.

15 LC-MS:(m-1)=787 (m/z)

(iii) (nPr)₂NCOCH₂OOCCH₂-(R)Cgl-Aze-Pab-OH

A solution of (nPr)₂NCOCH₂-(R)Cgl(Boc)-Aze-Pab-O-Boc (20mg; 0.025mmol) in TFA:methylene chloride (1:1) was stirred at room temperature for 5 minutes. The reaction mixture was concentrated and freeze dried from acetonitrile and water to give 5mg (34%) of the title compound.

LC-MS:(m+1)=587 (m/z)

25

Example 24

ChNHCOCH₂OOCCH₂-(R)Cgl-Aze-Pab-OH

The title compound was prepared analogously to the procedure described in Example 18 from ChNHCOCH₂OOCCH₂-(R)Cgl-Aze-Pab-Z (118mg; 0.17mmol; see Example 11(iii) above). Yield 1.8 mg.

30

LC-MS:($m+1$)=585 (m/z)

Example 25

MeNHCOCH₂OOCCCH₂-(R)Cgl-Aze-Pab-OH

- 5 The title compound was prepared analogously to the procedure described in Example 18 from MeNHCOCH₂-(R)Cgl-Aze-Pab-Z (81mg; 0.12mmol, see Example 36 below). Yield 10mg (16%).

LC-MS:($m+1$)=517 (m/z)

10

Example 26

EtOOCCCH₂-(R)Cgl-Aze-Pab-OAc

(i) H-(R)Cgl-Aze-Pab-OAc

- 15 The sub-title compound was prepared analogously to the method described in Example 27 below (steps (i), (ii) and (iii)) using acetic acid anhydride instead of propanoic acid anhydride.

LC-MS:($m+1$)=430 (m/z)

20

(ii) EtOOCCCH₂-(R)Cgl-Aze-Pab-OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(R)Cgl-Aze-Pab-OAc (370mg; 0.6mmol) and ethyl bromoacetate (105mg; 0.63mmol). Yield 67mg (22%).

25

LC-MS:($m+1$)=516 (m/z)

Example 27EtOOCCH₂-(R)Cgl-Aze-Pab-OC(O)Et(i) Boc-(R)Cgl-Aze-Pab-OH

- 5 To a solution of hydroxylamine hydrochloride and triethylamine in EtOH was added Boc-(R)Cgl-Aze-Pab-Z (1.0g; 1.52mmol). The reaction mixture was stirred at room temperature for 40 hours and then concentrated. The crude product was purified by preparative RPLC.

10 LC-MS: (m+1) = 488 m/z

(ii) Boc-(R)Cgl-Aze-Pab-OC(O)Et

- A solution of Boc-(R)Cgl-Aze-Pab-OH (500mg; 0.91mmol; from step (i) above) and propanoic acid anhydride (3.5 mL) was stirred at room temperature for 45 minutes and then concentrated. The crude product was purified by preparative RPLC using 50% acetonitrile in 0.1M NH₄OAc as eluent to give 266mg (54%) of the sub-title compound.
- 15

LC-MS:(m+1)= 544(m/z)

20

(iii) H-(R)Cgl-Aze-Pab-OC(O)Et

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from Boc-(R)Cgl-Aze-Pab-OC(O)Et (238mg; 0.44mmol; from step (ii) above). Yield 290mg (100%).

25

LC-MS:(m+1)= 444(m/z)

(iv) EtOOCCH₂-(R)Cgl-Aze-Pab-OC(O)Et

- To a solution of H-(R)Cgl-Aze-Pab-OOCET (300mg; 0.45mmol; from step (iii) above) and K₂CO₃ (308mg; 2.23mmol) in methylene chloride (6 mL)
- 30

at 0°C was added dropwise EtOOCCH₂OSO₂CF₃ (105mg; 0.45mmol, prepared from triflic anhydride and ethyl glycolate). After the reaction mixture was stirred at room temperature for 1 h the reaction mixture was washed with water, citric acid and water, dried (Na₂SO₄) and concentrated. The crude product was purified by preparative RPLC using 45% acetonitrile in 0.1M NH₄OAc as eluent to give 63mg (27%) of the title compound.

LC-MS:(m+1)= 530(m/z)

Example 28

HOOCCH₂-(R)Cgl-Aze-Pab-OH

(i) *t*BuOOCCH₂-(R)Cgl-Aze-Pab-OOCPh

The sub-title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-OOCPh (250mg; 0.5mmol; see Example 15(ii) above) and *t*-butylbromoacetate (119mg; 0.6mmol). Yield 211mg (69%).

LC-MS:(m+1)=606 (m/z)

(ii) HOOCCH₂-(R)Cgl-Aze-Pab-OOCPh

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from *t*BuOOCCH₂-(R)Cgl-Aze-Pab-OOCPh (233mg; 0.3mmol; from step (i) above). Yield 65mg (37%).

LC-MS:(m+1)=550 (m/z)

(iii) HOOCCH₂-(R)Cgl-Aze-Pab-OH

A solution of HOOCCH₂-(R)Cgl-Aze-Pab-OOCPh (60mg; 0.1mmol; from

step (ii) above) and KOMe (0.2M; 0.2mmol) in THF (10mL) and methanol (1.5mL) was stirred at room temperature for 5 minutes. The reaction mixture was concentrated and freeze dried from water and acetonitrile to give 28mg (63%) of the title compound.

5

LC-MS:(m+1) = 446(m/z)

Example 29

HOOCCH₂-(R)Cgl-Aze-Pab-O-cis-Oleyl

10

(i) *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-Z

A solution of *t*BuOOCCH₂-(R)Cgl-Aze-Pab-Z (1.7g, 2.8mmol; see Example 37 below), (Boc)₂O (672mg; 3.08mmol) and DMAP (68mg; 0.56mmol) in THF (30mL) was stirred at room temperature for 24 h.

15

Additional (Boc)₂O (305mg; 1.4mmol) was added at 5°C. After another 24 h the reaction mixture was concentrated and purified by preparative RPLC to give 587mg (30%) of the desired compound.

EC-MS:(m+1) = 720 (m/z)

20

(ii) *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-OH

The sub-title compound was prepared analogously to the procedure described in Example 18 from *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-Z (580mg; 0.8mmol; from step (i) above). Yield 341mg (71%).

25

EC-MS:(m+1) = 602 (m/z)

(iii) *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-O-cis-Oleyl

A solution of *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-OH (340mg; 0.56mmol; from step (ii) above), *cis*-oleylchloride (170mg; 0.56mmol) and triethylamine (62mg; 0.61mmol) in methylene chloride was stirred for 5

30

minutes. The reaction mixture was concentrated and purified by preparative RPLC to give 326mg (67%) of the sub-title compound.

EC-MS:(m+1)= 867(m/z)

5

(iv) HOOCCCH₂-(R)Cgl-Aze-Pab-O-cis-Oleyl

The title compound was prepared analogously to the procedure described in Example 1(ii) from *t*BuOOCCH₂-(R)Cgl(Boc)-Aze-Pab-O-cis-Oleyl (223mg; 0.25mmol; from step (iii) above).

10

LC-MS:(m+1)= 710 (m/z)

Example 30

Cyclooctyl-OOCCH₂-(R)Cgl-Aze-Pab-Z

15

(i) Cyclooctyl-bromoacetate

Cyclooctanol (1.3g; 10mmol) and DMAP (0.3g) was dissolved in methylene chloride followed by addition of bromoacetyl chloride (1mL; 12mmol). After stirring for 18 h the reaction mixture was washed with aqueous Na₂CO₃ (2M) and HCl (1M), dried, concentrated and purified by flash chromatography using petroleum ether:methylene chloride (50:50) to give 1.8g (72%) of the sub-title compound.

20

(ii) Cyclooctyl-OOCCH₂-(R)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z x 2HCl (703mg; 1.2mmol) and cyclooctyl bromoacetate (363mg; 1.46mmol; from step (i) above). Yield 379mg (46%).

25

30 FAB-MS:(m+1)= 674(m/z)

Example 31 $t\text{BuCH}_2\text{OOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z x 2HCl (2.5g; 4.3mmol) and
 5 tertbutylmethyl bromoacetate (1.08g; 5.2mmol). Yield 1.87g (69%).

FAB-MS:(m+1) = 634 (m/z)

Example 32

10 $(2\text{-Me})\text{BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

(i) Methylbenzyl bromoacetate

The sub-title compound was prepared analogously to the procedure described in Example 30(i) from 2-methylbenzylalcohol (5g; 41mmol) and
 15 bromoacetyl chloride (12.6g; 80mmol). Yield 8.2g (82%).

(ii) $(2\text{-Me})\text{BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z x 2HCl (580mg; 1mmol) and
 20 2-methylbenzyl bromoacetate (290mg; 1.2mmol; from step (i) above). Yield 30mg (4.5%).

LC-MS:(m+1) = 668 (m/z)

25 Example 33

$\text{ChCH}_2\text{OOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

A solution of BnOOCCH₂-(R)Cgl-Aze-Pab-Z (1.41g; 1.7mmol) and cyclohexyl methylalcohol (6mL) in triethylamine (474 μL) and methylene chloride (3mL) was refluxed for 4 days. The reaction mixture was worked
 30 up to give a crude product which was purified by flash chromatography—

using methylene chloride:methanol (95:5) as eluent to give 801mg (71%) of the title compound.

FAB-MS:(m+1)= 660 (m/z)

5

Example 34

ChOOCCH₂-(R)Cgl-Aze-Pab-Z

(i) Cyclohexyl bromoacetate

- 10 The sub-title compound was prepared analogously to the procedure described in Example 32(i) above from cyclohexanol (1g; 10mmol) and bromoacetylchloride (1mL; 12mmol).

(ii) ChOOCCH₂-(R)Cgl-Aze-Pab-Z

- 15 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z x 2HCl (2.5g; 4.32mmol) and cyclohexyl bromoacetate (1.5g; 5.2mmol). Yield 1.7g (60%).

FAB-MS:(m+1)= 646 (m/z)

20

Example 35

PhC(Me)₂OOCCH₂-(R)Cgl-Aze-Pab-Z

(i) 2-Phenyl-2-propyl bromoacetate

- 25 The sub-title compound was prepared analogously to the procedure described in Example 30(i) from 2-phenyl-2-propanol (3g; 22mmol) and bromoacetylchloride (4.16g, 26mmol). Yield 1.2g (44%).

(ii) PhC(Me)₂OOCCH₂-(R)Cgl-Aze-Pab-Z

- 30 The title compound was prepared analogously to the procedure described

in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (1.2g; 2.2mmol) and 2-phenyl-2-propyl bromoacetate (640mg; 2.5mmol; from step (i) above). Yield 1.3g (86%).

- 5 ¹H-NMR (500 MHz; CDCl₃) δ 9.3 (br s, 1H), 8.35 (t, 1H), 7.75 (d, 2H), 7.45 (d, 2H), 7.30-7.05 (m, 10H or 11H), 5.15 (s, 2H), 4.78 (t, 1H), 4.40-4.30 (AB part of ABX spectrum, 2H), 3.95 (q, 1H), 3.74 (q, 1H), 3.27-3.19 (AB-spectrum, 2H), 2.72 (d, 1H), 2.43 (q, 2H), 1.93 (br d, 1H), 1.75-1.60 (m, 9H or 10H), 1.54 (d, 1H), 1.49-1.40 (m, 1H), 1.25-1.0 (m, 4H), 0.92 (q, 1H)
- 10

Example 36

MeNHCOCH₂OOCCH₂-(*R*)Cgl-Aze-Pab-Z

- The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (1.0g; 1.7mmol) and MeNHCOCH₂OOCCH₂Br (440mg; 2mmol; prepared analogously to the procedures described in Example 11 above (steps (i), (ii) and (iii)) using methylamine instead of cyclohexylamine). Yield 380mg (35%).
- 15

- 20 FAB-MS:(m+1)= 635 (m/z)

Example 37

*t*BuOOCCH₂-(*R*)Cgl-Aze-Pab-Z

- The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(*R*)Cgl-Aze-Pab-Z x 2HCl (500mg; 1.0mmol) and *t*-butyl bromoacetate (231mg; 1.2mmol). Yield 420mg (69%).
- 25

LC-MS:(m+1)= 620 (m/z)

Example 38(Me)₂CHC(Me)₂OOCCH₂-(R)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (787mg; 1.4mmol) and 2,3-dimethyl-2-butyl bromoacetate (364mg; 1.63mmol). Yield 590mg (67%).

FAB-MS:(m+1) = 648(m/z)

Example 3910 iPrOOCCH₂-(R)Cgl-Aze-Pab-Z

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (700mg; 1.2mmol) and isopropyl bromoacetate (262mg; 1.5mmol). Yield 225mg (31%)

15 FAB-MS:(m+1) = 606(m/z)

Example 40BnOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)20 (i) Boc-(R)Cgl-Aze-Pab-COOPh(4-OMe)

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab-H and 4-methoxyphenyl chloroformate.

25 FAB-MS:(m+1) = 622(m/z)

(ii) H-(R)Cgl-Aze-Pab-COOPh(4-OMe) x 2HCl

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(R)Cgl-Aze-Pab-COOPh(4-OMe)

30 (from step (i) above).

(iii) BnOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOPh(4-OMe) x 2HCl (85mg; 0.16mmol; from step (iii) above) and benzyl bromoacetate (90mg; 0.2mmol). Yield 60mg (56%).

FAB-MS:(m+1)= 670 (m/z)

Example 4110 ChCH₂OOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOPh(4-OMe) (554mg; 0.64mmol; see Example 40(ii) above) and cyclohexylmethyl bromoacetate (165mg; 0.7mmol). Yield 34mg (8%).

15

FAB-MS:(m+1)= 676 (m/z)

Example 42(2-Me)BnOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)

20 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOPh(4-OMe) (522mg; 1mmol; see Example 40(ii) above) and 2-(methyl)benzyl bromoacetate (365mg; 1.5mmol). Yield 158mg (23%).

25 LC-MS:(m+1)= 684 (m/z)

Example 43EtOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-Me)(i) Boc-(R)Cgl-Aze-Pab-COOPh(4-Me)

- 5 The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab (1.96g; 4.56mmol) and 4-tolyl-chloroformate (850mg; 4.99mmol). Yield 1.39g (55%).

FAB-MS:(m+1) = 606(m/z)

10

(ii) H-(R)Cgl-Aze-Pab-COOPh(4-Me)

The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(R)Cgl-Aze-Pab-COOPh(4-Me) (388mg; 0.64mmol; from step (i) above). Yield 293mg (91%).

15

FAB-MS:(m+1) = 506(m/z)

(iii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-Me)

- 20 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOPh(4-Me) (288mg; 0.6mmol; from step (ii) above) and ethyl bromoacetate (114mg; 0.7mmol). Yield 81mg (24%).

FAB-MS:(m+1) = 592 (m/z)

25

Example 44BnOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-Me)

- The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOPh(4-Me) (272mg; 30 0.54mmol; see Example 43(ii) above) and benzyl bromoacetate (147mg;

0.6mmol). Yield 107mg (31%).

FAB-MS: $(m+1) = 654 (m/z)$

5 Example 45

BnOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-*n*Bu x 2HCl (400mg; 0.74mmol; see Example 9(ii) above) and benzyl bromoacetate (210mg; 0.88mmol). Yield 220mg (48%).

FAB-MS: $(m+1) = 620 (m/z)$

Example 46

15 *i*PrOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂CH=CH₂ x 2TFA (456mg; 0.67mmol; see Example 1(ii) above) and isopropyl bromoacetate (145mg; 0.8mmol). Yield 294mg (79%).

20

FAB-MS: $(m+1) = 556 (m/z)$

Example 47

EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*i*Bu

25

(i) Boc-Pab-COO-*i*Bu

To a solution of Boc-Pab-H (500mg; 2.0 mmol; prepared from Pab-Z and (Boc)₂O (forming Boc-Pab-Z), followed by hydrogenation over Pd/C) and triethylamine (400mg; 4.0mmol) in methylene chloride (10mL) was added *i*-butyl chloroformate (270mg; 2.2mmol) at 0°C. After stirring for 5 h,

30

water was added. The organic phase was dried (Na_2SO_4) and concentrated to give 530mg (76%) of the sub-title compound.

^1H -NMR (500 MHz, CDCl_3) δ 9.5 (bs, 1H), 7.82 (d, 2H), 7.31 (d, 2H),
5 6.6 (bs, 1H), 5.0 (bs, 1H), 4.33 (bd, 2H), 3.93 (d, 2H), 2.04 (m, 1H),
1.45 (s, 9H), 0.97 (d, 6H)

(ii) H-Pab-COO-*i*Bu x 2HCl

The sub-title compound was prepared analogously to the procedure
10 described in Example 4(ii) from Boc-Pab-COO-*i*Bu (520mg; 1.5mmol;
from step (i) above). Yield 430mg (88%).

^1H -NMR (500 MHz, MeOD) δ 7.89 (d, 2H), 7.75 (d, 2H), 4.30 (s, 2H),
15 4.17 (d, 2H), 2.11-2.05 (m, 1H), 1.02 (d, 6H)

(iii) Boc-(*R*)Cgl-Aze-Pab-COO-*i*Bu

To a solution of Boc-(*R*)Cgl-Aze-OH (480mg; 1.4mmol), H-Pab-COO-*i*Bu
x 2HCl (430mg; 1.3mmol; from step (ii) above) and DMAP (650mg;
5.3mmol) in acetonitrile (20mL) was added EDC (270mg; 1.4mmol).
20 After stirring for 3 days at room temperature the reaction mixture was
concentrated and then dissolved in water and EtOAc. The organic phase
was washed with NaHCO_3 (aq) and dried (Na_2SO_4), concentrated and
purified by flash chromatography using EtOAc as eluent to give 510mg
(52%) of the sub-title compound.

(iv) H-(*R*)Cgl-Aze-Pab-COO-*i*Bu x 2HCl

The sub-title compound was prepared analogously to the procedure
described in Example 4(ii) from Boc-(*R*)Cgl-Aze-Pab-COO-*i*Bu (500mg;
0.88mmol; from step (iii) above). Yield 360mg (87%).

(v) EtOOCCH₂-(R)Cgl-Aze-Pab-COO-*i*Bu

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-*i*Bu x 2HCl (290mg; 0.53mmol; from step (iv) above) and ethyl bromoacetate (110mg; 0.64mmol). Yield 140mg (47%).

FAB-MS:(m+1) = 558 (m/z)

Example 4810 BnOOCCH₂-(R)Cgl-Aze-Pab-COO-*n*Pr

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-*n*Pr x 2TFA (902mg; 1.3mmol; see Example 5(ii) above) and benzyl bromoacetate (362mg; 1.6 mmol). Yield 199mg (25%).

15

¹H-NMR: (400MHz; CDCl₃) δ 8.43 (bs, 1H), 7.78 (d, 2H), 7.38-7.27 (m, 7H), 5.05 (s, 2H), 4.90 (dd, 1H), 4.56-4.39 (AB part of ABX spectrum, 2H), 4.12-4.03 (m, 3H), 3.98-3.91 (q, 1H), 3.33-3.22 (AB-spectrum, 2H), 2.85 (d, 1H), 2.65-0.94 (m, 19H)

20

Example 49EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCCh(i) EtSCOOCH₂OOCCh

25 To a solution of tetrabutylammonium hydrogensulphate (15.6g, 45.6mmol) and cyclohexane carboxylic acid (5.85g, 46mmol) in methylene chloride was added NaOH (9.1mL, 10M; 68mmol) at 0°C. After stirring for 5 minutes the reaction mixture was filtered, washed with methylene chloride, dissolved in toluene, concentrated and dissolved in THF to give
30 [Bu₄N]⁺[OOCCh]⁻. EtSCOOCH₂Cl (4g; 25.9mmol; see Folkmann and

Lund, J. Synthesis, (1990), 1159) was added to the THF solution of $[\text{Bu}_4\text{N}]^+[\text{OOCCh}]^-$ at room temperature. After stirring at room temperature for 12 h the reaction mixture was concentrated and purified by flash chromatography to give 2.57g (40%) of the sub-title compound.

5

$^1\text{H-NMR}$ (400 MHz, CDCl_3) diagnostic peaks δ 5.80 (s, 2H, O- CH_2 -O), 2.85 (q, 2H, CH_2 -S)

(ii) $\text{ClCOOCH}_2\text{OOCCh}$

10 To $\text{EtSCOOCH}_2\text{OOCCh}$ (2.9g; 11.8mmol; from step (i) above) was added dropwise SO_2Cl_2 (3.18g; 23.6mmol) at 0°C . After stirring for 30 minutes the reaction mixture was concentrated to give 1.82g (70%) of the desired compound.

15 $^1\text{H-NMR}$ (500 MHz, CDCl_3) diagnostic peaks δ 5.82 (s, 2H, O- CH_2 -O)

(iii) $\text{Boc-(R)Cgl-Aze-Pab-COOCH}_2\text{OOCCh}$

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from $\text{Boc-(R)Cgl-Aze-Pab-H}$ (750mg; 20 1.59mmol) and $\text{ClCOOCH}_2\text{OOCCh}$ (460mg; 2.1mmol; from step (ii) above). The crude product was purified by preparative RPLC. Yield 355mg (9%).

FAB-MS: $(m+1) = 656(m/z)$

25

(iv) $\text{H-(R)Cgl-Aze-Pab-COOCH}_2\text{OOCCh} \times 2\text{TFA}$

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from $\text{Boc-(R)Cgl-Aze-Pab-COOCH}_2\text{OOCCh}$ (from step (iii) above).

30

(v) EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCCH₂

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂OOCCH₂ x 2TFA (193mg; 0.35mmol; from step (iv) above) and ethyl trifluoroacetate
 5 (83mg; 0.35mmol). Yield 87mg (39%).

¹H-NMR (400 MHz, CDCl₃) δ 8.48 (t br, 1H), 7.83 (d, 2H), 7.37 (d, 2H), 5.86 (s, 2H), 4.95 (dd, 1H), 4.15-4.39 (AB part of ABX spectrum, 2H), 4.18-4.05 (m, 5H), 3.26-3.17 (AB-spectrum, 2H), 2.87 (d, 1H),
 10 2.75-0.95 (m, 29H)

Example 50EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCCH₂Ch

The title compound was prepared analogously to the procedure described
 15 in Example 49 above starting with cyclohexyl acetic acid instead of cyclohexane carboxylic acid. Yield 74mg (17%).

FAB-MS:(m+1) = 656(m/z)

20 Example 51EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH(Me)OOCPh

The title compound was prepared analogously to the procedure described in Example 49 above starting with EtSCOOCH(CH₃)Cl (prepared from ClCOCH(CH₃)Cl and EtSH using the procedure described by Folkmann
 25 *et al* in J. Synthesis, (1990), 1159) instead of EtSCOOCH₂Cl. Yield 70mg (23%).

FAB-MS:(m+1) = 650 (m/z)

Example 52EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOCPh

The title compound was prepared analogously to the procedure described in Example 49 above using benzoic acid instead of cyclohexane carboxylic acid. Yield 50mg (39%).

¹H-NMR (300 MHz, CDCl₃) δ 9.73-9.25 (s br, 1H), 8.45 (t, 1H), 8.05 (d, 2H), 7.83 (d, 2H), 7.60-7.10 (m, 6H), 6.10 (s, 2H), 4.96-4.84 (dd, 1H), 4.62-4.30 (ABX, 2H), 4.20-3.93 (m, 4H), 3.25 (s, 2H), 2.84 (d, 1H), 2.73-2.41 (m, 2H), 2.41-0.87 (m, 15H)

¹³C-NMR (300 MHz, CDCl₃, amidine and carbonyl carbons) δ 163.1, 165.3, 169.0, 170.8, 172.3, 175.5

Example 53BnOOCCH₂-(R)Cgl-Aze-Pab-COOCH(Me)OAc

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH(CH₃)OC(O)CH₃ (108mg; 0.21mmol; see Example 14(ii) above) and benzyl bromoacetate (36μL; 0.23mmol). Yield 41mg (30%).

FAB-MS:(m+1)= 650(m/z)

Example 54EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OAc(i) H-(R)Cgl-Aze-Pab-COOCH₂OAc x 2TFA

The sub-title compound was prepared analogously to the procedure described in Example 14 (steps (i) and (ii)) above using acetoxymethyl 4-nitrophenylcarbonate (prepared analogously to the method described in Example 12(iii) using silver acetate instead of silver pivalate). Work up

gave the sub-title compound which was used in the next step without further purification.

(ii) EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OAc

- 5 The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(R)Cgl-Aze-Pab-COOCH₂OAc x 2TFA (0.83mmol; from step (i) above) and ethyl bromoacetate (2.2mmol). Yield 286mg.

10 FAB-MS:(m+1) = 574 (m/z)

Example 55

tBuOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OAc

- 15 The title compound was prepared analogously to the procedure described in Example 1(iii) above from H-(R)Cgl-Aze-Pab-COOCH₂OAc x 2TFA (0.313mmol; see Example 54(i) above) and *t*-butyl bromoacetate (73mg; 0.376mmol). Yield 156mg (83%).

FAB-MS:(m+1) = 602(m/z)

20

Example 56

BnOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂OOC-*t*Bu

- 25 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂OOC-*t*Bu (379mg; 0.71mmol; see Example 12(v) above) and benzyl bromoacetate (135μL; 0.85mmol). Yield 146mg (30%).

FAB-MS:(m+1) = 678(m/z)

Example 57EtOOCCH₂-(R)Cgl-Aze-Pab-COOCH₂CCl₃(i) Boc-(R)Cgl-Aze-Pab-COOCH₂CCl₃

- 5 The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab-H (1.0g; 2.12 mmol), 2M NaOH (11.7 ml) and trichloroethyl chloroformate (494 mg; 2.33 mmol). Yield 1.08g (79%).

10 (ii) H-(R)Cgl-Aze-Pab-COOCH₂CCl₃

The sub-title compound was prepared analogously to the procedure described in Example 1(ii) from Boc-(R)Cgl-Aze-Pab-COOCH₂CCl₃ (1.04g; 1.607 mmol; from step (i) above). Yield 1.43g (99%).

- 15 ¹H-NMR: (500MHz; CD₃OD) δ 7.79 (d, 2H), 7.61 (d, 2H), 5.10 (s, 2H), 4.87-4.81 (m, 2H), 4.63-4.52 (q, 2H), 4.41-4.34 (m, 1H), 4.30-4.24 (m, 1H), 3.72 (d, 1H), 2.72-2.63 (m, 1H), 2.32-2.25 (m, 1H), 1.88-1.10 (m, 14H)

20 (iii) EtOOCCH₂-(R)Cgl-Aze Pab-COOCH₂CCl₃

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂CCl₃ (400 mg; 0.52 mmol; from step (ii) above) and ethyl bromoacetate (95 mg; 0.57 mmol). Yield 8 mg (23%).

25

¹H-NMR: (500MHz; CDCl₃) δ 8.47 (bt, 1H), 7.83 (d, 2H), 7.48 (bs, 1H), 7.31 (d, 2H), 4.92 (dd, 1H), 4.85 (s, 2H), 4.58-4.39 (AB part of ABX spectrum, 2H), 4.16-4.06 (m, 4H), 3.24 (s, 2H), 4.87 (d, 1H), 2.65-2.59 (m, 1H), 2.56-2.48 (m, 1H), 2.10-0.95 (m, 16H)

30

Example 58MeOOC-C(=CEt)CH₂OOCCCH₂-(R)Cgl-Aze-Pab-Z(i) MeOOC-C(=CH)C(OH)Et

- 5 Propionaldehyde (10.1 g; 0.174 mol) was added dropwise to a solution of methyl acrylate (10g; 0.116mol) and 1,4-diazobicyclo[2,2,2]octane (1.3 g; 0.0116 mol). The reaction mixture was stirred at room temperature for 14 days. Ethyl acetate (150 ml) was added. The organic phase was washed with water and brine, dried (Na₂SO₄), filtered and concentrated to give the
- 10 desired compound. Yield 15.5g (93%).

¹H-NMR: (400MHz; CDCl₃) δ 6.24 (s, 1H), 5.81 (s, 1H), 4.34 (t, 1H), 3.78 (s, 3H), 2.82 (bs, 1H), 1.69 (m, 2H), 0.95 (t, 3H)

15 (ii) MeOOC-C(=CEt)CH₂Br

- HBr (6.5 ml, ~48%) was added dropwise to MeOOC-C(=CH)C(OH)Et (3 g; 20.8 mmol; from step (i) above) at 0°C. After 5 minutes H₂SO₄ (conc.; 6 ml) was added dropwise. The reaction mixture was stirred for 12 hours at room temperature. Two phases was separated and the top
- 20 phase was diluted with ether. The ether phase was washed with water and aqueous NaHCO₃, dried (Na₂SO₄ and charcoal) and concentrated. The residue was purified by flash chromatography. Yield 1.7g (40%).

- ¹H-NMR: (400MHz; CDCl₃) δ 6.97 (t, 3H), 4.23 (s, 2H), 3.8 (s, 3H),
- 25 2.32 (m, 2H), 1.13 (t, 3H)

(iii) tBuOOCCCH₂-(R)Cgl-Aze-Pab-Z

- The sub-title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (2.1 g; 3.6 mmol)
- 30 and *t*-butyl bromoacetate (780 mg; 4.0 mmol). Yield 1.73g (78%).

(iv) HOOCCH₂-(R)Cgl-Aze-Pab-Z

A solution of *t*BuOOCCH₂-(R)Cgl-Aze-Pab-Z (from step (iii) above) and TFA in methylene chloride was stirred in room temperature for 3 h. The reaction mixture was concentrated and freeze dried from water and HCl (conc.; 10 eq.).

(v) MeOOC-C(=CEt)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z

A solution of HOOCCH₂-(R)Cgl-Aze-Pab-Z (263 mg; 0.41 mmol; from step (iv) above), NaOH (1M; 1.239 ml; 1.239 mmol) and water (4 ml) was freeze dried. DMF (5 ml) was added, followed by dropwise addition of Me-OOC-C(=CEt)CH₂Br (103 mg; 0.496 mmol; from step (ii) above) at 0°C. The reaction mixture was stirred for 24 h at room temperature, diluted with toluene (5 ml), washed with water, dried (Na₂SO₄) and concentrated. The residue was purified by flash chromatography using EtOAc:methanol (95:5) as eluent. Yield 95 mg (33%).

FAB-MS:(m+1) = 690 (m/z)

Example 5920 MenOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)(i) MenOOCCH₂Br

The sub-title compound was prepared analogously to the procedure described in Example 30(i) above from MenOH (10 mmol) and bromoacetyl chloride (12 mmol). Yield 1.5g (54%).

(ii) MenOOCCH₂-(R)Cgl-Aze-Pab-COOPh(4-OMe)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-Ph(4-OMe) (521 mg; 1 mmol; see Example 40(ii) above) and MenOOCCH₂Br (416 mg; 1.5

mmol; from step (i) above). Yield 36 mg (5%).

FAB-MS: $(m+1) = 718$ (m/z)

5 Example 60

$t\text{BuOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOnPr}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-*n*Pr (575 mg; 0.837 mmol; see Example 5(ii) above) and *t*-butyl bromoacetate (196 mg; 1.01 mmol). Yield 110 mg (23%).

LC-MS: $(m+1) = 572$ (m/z)

Example 61

15 $\text{MenOOCCH}_2\text{-(R)Cgl-Aze-Pab-Z}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (0.7 g; 1.21 mmol) and MenOOCCH₂Br (0.4 g; 1.45 mmol; see Example 59(i) above). Yield 0.33g (38%).

20

FAB-MS: $(m+1) = 702$ (m/z)

Example 62

$\text{BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-COO-Bn(4-NO}_2\text{)}$

25

(i) $\text{Boc-(R)Cgl-Aze-Pab-COO-Bn(4-NO}_2\text{)}$

The sub-title compound was prepared analogously to the procedure described in Example 1(i) from Boc-(R)Cgl-Aze-Pab-H (1.03g; 2.18 mmol), 2M NaOH (24 mL) and 4-NO₂-benzyl chloroformate (518 mg; 2.4 mmol). Yield 1.32g (93%).

30

FAB-MS: $(m+1)=651(m/z)$

(ii) H-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂)

- The sub-title compound was prepared analogously to the procedure described in Example 4(ii) from Boc-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂) (1.32 mg; 2.03 mmol; from step (i) above). Yield 1.0g (79%).

FAB-MS: $(m+1)=551(m/z)$

10 (iii) BnOOCCH₂-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂) (0.5g; 0.80mmol; from step (ii) above) and benzyl bromoacetate (220 mg; 0.90 mmol).

15

FAB-MS: $(m+1)=699(m/z)$

Example 63

EtOOCCH₂-(R)Cgl-Aze-Pab-Bn(4-NO₂)

- 20 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂) (211 mg; 0.38mmol; see Example 62(ii) above) and ethyl bromoacetate (47 μ l; 0.42 mmol). Yield 44 mg (18%).

- 25 ¹H-NMR: (300MHz; CDCl₃) δ 9.55 (bs, 1H), 8.50 (bt, 1H), 8.20 (d, 2H), 7.80 (d, 2H), 7.60 (d, 2H), 7.35 (d, 2H), 6.87 (bs, 1H), 4.95 (dd, 1H), 4.65-4.40 (AB part of ABX spectrum, 2H), 4.18-4.04 (m, 5H), 3.27-3.15 (AB-spectrum, 2H), 2.87 (d, 1H), 2.75-2.60 (m, 1H), 2.57-2.45 (m, 1H), 2.00-0.95 (m, 16H).

30

Example 64PrIc(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z(i) PrIc(O)CH₂OH

- 5 A mixture of 2,5-dioxo-1,4-dioxane (2.0 g; 17 mmol) and pyrrolidine (8 ml; 97 mmol) was refluxed for 1 h. The excess pyrrolidine was removed by evaporation. Yield 4.4g (99%).

FAB-MS(*m*+1)=130(*m/z*)

10

(ii) PrIc(O)CH₂OOCCH₂Br

- To a solution of PrIc(O)CH₂OH (0.4 g; 3.1 mmol; from step (i) above) in DMF (15 ml) was added dropwise bromoacetyl bromide (0.63 g; 3.1 mmol) at 0°C. The reaction mixture was stirred for 1.5 h at 0°C and 3 h
15 at room temperature. Additional bromoacetyl bromide (0.63 g; 3.1 mmol) was added and the reaction mixture was heated to 80°C, stirred at room temperature for 12 h and concentrated. Yield 320 mg (41%)

FAB-MS(*m*+1)=252(*m/z*)

20

(iii) PrIc(O)CH₂OOCCH₂-(R)Cgl-Aze-Pab-Z

- The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-Z (580 mg; 1 mmol) and PrIc(O)CH₂OOCCH₂Br (300 mg; 1.2 mmol; from step (ii) above). Yield
25 400 mg (60%).

FAB-MS(*m*+1)=675 (*m/z*)

- ¹H-NMR: (500MHz; CDCl₃) δ 9.66-9.42 (bs, 1H), 8.64-8.56 (m, 1H), 8.03-7.93 (d, 2H), 7.89-7.66 (bs, 1H), 7.45 (d, 2H), 7.45-7.25 (m, 5H),
30 5.20 (s, 2H), 4.98-4.92 (dd, 1H), 4.82-4.74 (m, 1H), 4.62, 4.58 (AB

spectrum, 2H), 4.26-4.05 (m, 3H), 3.47-3.16 (m, 6H), 2.95 (d, 1H), 2.78-2.68 (m, 1H), 2.54-2.42 (m, 1H), 2.03-1.95 (m, 16H)

Example 65

5 (2-Me)BnOOCCH₂-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂)

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COO-Bn(4-NO₂) (500 mg; 0.80mmol; see Example 62(ii) above) and 2-(methyl)benzyl bromoacetate (234 mg; 0.96 mmol; see Example 32(i) above). Yield 528 mg (92%).

10

¹H-NMR: (400MHz, CDCl₃) δ 9.34 (bs, 1H), 8.38 (t, 1H), 8.09 (d, 2H), 7.72 (d, 2H), 7.48 (d, 2H), 7.37 (bs, 1H), 7.23 (d, 2H), 7.17-7.05 (m, 4H), 5.18 (s, 2H), 5.00 (s, 2H), 4.81 (dd, 1H), 4.45-4.34 (AB part of ABX spectrum, 2H), 4.04-3.97 (q, 1H), 3.93-3.86 (q, 1H), 3.27-3.17 (AB spectrum, 2H), 2.79 (d, 1H), 2.54-2.35 (m, 2H), 2.22 (s, 3H), 1.91-1.84 (bd, 1H), 1.71-1.39 (m, 5H), 1.19-0.84 (m, 4H).

15

Example 66

MeOOCCH₂-(R)Cgl-Aze-Pab-COOEt

20 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOEt (305 mg; 0.69 mmol; see Example 4(ii) and methyl bromoacetate (126 mg; 0.83 mmol). Yield 188 mg (53%).

25 LC-MS:(m+1)=516(m/z)

Example 67 $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OOCCH}_2\text{-(R)Cgl-Aze-Pab-COO-Bn(4-NO}_2\text{)}$ (i) $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OOCCH}_2\text{Cl}$

- 5 A mixture of $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OH}$ (244 mg; 1.53 mmol; see Example 12(i) above) and bromoacetyl chloride (270 mg; 1.72 mmol) was stirred at room temperature for 12 hours. The mixture was poured into aqueous NaHCO_3 and extracted with methylene chloride. The organic phase was washed with aqueous KHSO_4 (0.2M) and brine, dried and concentrated.

10

FAB-MS: $(m+1)=237(m/z)$ $^1\text{H-NMR}$: (400MHz, CDCl_3) δ 4.82 (s, 2H), 4.22 (s, 2H), 3.31-3.26 (t, 2H), 3.10-3.15 (t, 2H), 1.68-1.52 (m, 2H), 1.97-0.86 (m, 6H)

- 15 (ii) $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OOCCH}_2\text{-(R)Cgl-Aze-Pab-COO-Bn(4-NO}_2\text{)}$

The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOBn(4-NO₂) (343 mg; 0.62mmol; see Example 62(ii) above) and $(n\text{Pr})_2\text{NC}(\text{O})\text{CH}_2\text{OOCCH}_2\text{Cl}$ (160 mg; 0.68 mmol; from step (i) above). Yield 89 mg (19%).

20

FAB-MS: $(m+1)=750(m/z)$ Example 68 $(2\text{-Me)BnOOCCH}_2\text{-(R)Cgl-Aze-Pab-COOCH}_2\text{OOCtBu}$

- 25 The title compound was prepared analogously to the procedure described in Example 1(iii) from H-(R)Cgl-Aze-Pab-COOCH₂OOCtBu (380 mg; 0.71 mmol; see Example 12(v) above) and 2-(methyl)benzyl bromoacetate (215 mg; 0.88 mmol; see Example 32(i) above). Yield 37 mg (7.5%).

- 30 FAB-MS: $(m+1)=692(m/z)$

Example 69

The compounds of Examples 1 to 68 were all tested in Test A above and were all found to exhibit an $IC_{50}TT$ value of more than $1.0\mu M$ (ie they were are inactive to thrombin *per se*; cf. the active inhibitor $HOOC-CH_2-$
 5 $(R)Cgl-Aze-Pab-H$ which exhibits an $IC_{50}TT$ of $0.01\mu M$).

Example 70

The compounds of Examples 1 to 68 were tested in one, two or all of Tests B, C and/or D above, and were all found to exhibit oral and/or
 10 parenteral bioavailability in the rat as the active inhibitor $HOOC-CH_2-(R)Cgl-Aze-Pab-H$, either as the free acid and/or as one or more ester thereof. Based on the assumption that $HOOC-CH_2-(R)Cgl-Aze-Pab-H$ is formed in the rat, the bioavailability was calculated according to the formulae described in Test B and/or Test C as appropriate.

15

Abbreviations

	Ac =	acetyl
	aq =	aqueous
20	Aze =	S-Azetidine-2-carboxylic acid
	Boc =	<i>t</i> -butyloxycarbonyl
	$(Boc)_2O =$	di- <i>t</i> -butyldicarbonate
	Bn =	benzyl
	Bu =	butyl
25	Cgl =	cyclohexylglycine
	Ch =	cyclohexyl
	DCC =	dicyclohexyl carbodiimide
	DMAP =	N,N-dimethyl amino pyridine
	EDC =	1-(3-dimethylaminopropyl)-3-ethylcarbodiimide
30		hydrochloride

	Et =	ethyl
	EtOH =	ethanol
	EtOAc =	ethyl acetate
	h =	hours
5	HCl =	hydrochloric acid
	H-Pab-H =	1-amidino-4-aminomethyl benzene
	H-Pab-Z =	4-aminomethyl-1-(N-benzyloxycarbonylamidino) benzene
	HPLC =	high performance liquid chromatography
10	K ₂ CO ₃ =	anhydrous potassium carbonate
	Me =	methyl
	Men =	(1 <i>R</i> ,2 <i>S</i> ,5 <i>R</i>)-menthyl
	Pab-OH =	4-aminomethyl-benzamidoxime (4-aminomethyl-1-(amino-hydroxyiminomethyl) benzene
15	Piv(aloyl) =	2,2-dimethylacetyl
	Pr =	propyl
	Prl =	N-pyrrolidinyl
	RPLC =	reverse phase high performace liquid chromatography
20	TFA =	trifluoroacetic acid
	THF =	tetrahydrofuran
	Z =	benzyloxy carbonyl

Prefixes *n*, *s*, *i* and *t* have their usual meanings: normal, iso, sec and
 25 tertiary. Prefixes in the NMR-spectra *s*, *d*, *t*, *q*, and *b* mean singlet,
 doublet, triplet, quartet, and broad, respectively. The stereochemistry for
 the amino acids is by default (*S*) if not otherwise stated.